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# ANNALS OF BOTANY

VOL. XIV



Oxford

PRINTED AT THE CLARENDON PRESS

BY HORACE HART, M.A.

PRINTER TO THE UNIVERSITY







*Richard Spruce*  
Yours very faithfully  
*Richard Spruce.*

# ANNALS OF BOTANY

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VOLUME XIV

London

HENRY FROWDE, M.A., AMEN CORNER, E.C.

OXFORD: CLARENDON PRESS DEPOSITORY, 116 HIGH STREET

1900



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## ERRATUM.

P. 459, line 15 from top, for *cortex* read *phloem*.

## RICHARD SPRUCE.

(*With Portrait.*)

THE life of Richard Spruce impresses one as that of a man from whom the scientific world did not obtain all that he might have given. That it was not otherwise may be attributed to the demon of ill-health which overshadowed him from an early period in life, and which, if it determined the sphere of his work during that portion of his career which was active, crippled him as an acute sufferer during the later half. In all the circumstances we can but wonder that he accomplished so much.

The story of his life is in brief:—Born a Yorkshireman, at Ganthorpe, in 1817, Spruce having a mathematical gift followed the profession of his father, and became for a short period a dominie. But his unquenchable love of natural history, more especially of the Bryophyta, backed probably by a temperament unsuited to the schoolroom, ere long weaned him from the desk, and the calls of health imperatively demanding his sojourn in a climate less rigorous than that of Britain, he spent a short year in botanical exploration upon the Pyrenees, and subsequently migrated to South America upon a like errand with the support of the leading British botanists of the time. Landing at Para, in 1849, he began a series of explorations and botanical collections which employed him for a dozen years, in course of which he traversed South America by divergent routes from East to West, passing but a few degrees northwards or southwards of the equator over a region—including many important rivers, some of them up to that time unexplored—extending from British Guiana and Venezuela on the north-east to Peru on the south-west. In



addition to his botanical zeal which enabled him to reap a rich harvest of plants which were from time to time transmitted to Europe for distribution and sale for his support, Spruce's skill as a cartographer enabled him to add materially to our knowledge of the geography of South America. When at Ambato, on the Quitensian Andes, in 1859, his services were secured as one of the band under Mr. Clements Markham, to which, in imitation of the example set by the Dutch, was entrusted the work of securing plants and seeds of *Cinchona* for transmission to India—work which was to lay the foundation of the important enterprise which has been so successful and so beneficial. To Spruce the chief duty of looking after the supply of *Cinchona succirubra* from the slopes of Chimborazo devolved, and his mission was accomplished successfully in so far as the *Cinchona* plants were concerned, but disastrously for himself. Early in 1860, during its progress, his health entirely broke down, and thus maimed he made his way to Guayaquil, only to find himself financially ruined by fraud. A couple of years more concluded his fifteen years' life in South America, and he returned to England. A small Government-pension relieved his straitened circumstances to some extent, and this was increased subsequently by a belated grant from the Government of India in recognition of his services in connexion with *Cinchona*. Settling first at Hurstpierpoint, Spruce then moved to Welburn in Yorkshire, and ultimately to Coneysthorpe, where he died December 28, 1893, having by careful regimen prolonged his life to the advanced age of seventy-six years.

The botanical work of Spruce falls into two distinct periods, leaving out of account his few early essays. The first, that of his American travels, credits him with an enormous increase to our knowledge of plant-forms through the collections he poured into Europe, estimated to include some seven thousand species of Flowering Plants and Ferns, besides a vast number of lower Cryptogamia, especially of Mosses and Liverworts. Few travellers have ever collected so extensively, and with so much judgement and skill; his neatness of hand and

methodical habits peculiarly suited his work. In addition to this provision of these material accessions to our knowledge of the Vegetable Kingdom, Spruce's service to the cause of humanity in his *Cinchona* work must always be reckoned as of his greatest. Sojourn in the American forests, whilst it fascinated did not stimulate him to be a dreamer of scientific dreams as it did his fellow traveller Wallace, and Bates and Belt; or, if it did, he did not write the interpretation. The few letters of his fitfully describing his progress to Sir William Hooker, Mr. Bentham, and other botanists, which appeared in the *Kew Journal of Botany* in the fifties, and which are marked by a clear and facile diction, show him as a shrewd observer, and as a naturalist alive to biological problems wanting solution for which the materials might be at his hand. But he never achieved any compendious account of his travels, through which he might have conveyed the impressions of his long American experience, and the glimpses that his writings give us of the keenness of his perception and of his critical faculty indicate that Science is in this a loser.

The second period—that in England of the latter half of his life, when, as he says, he was 'never more able to sit straight up or walk about without great pain or discomfort,' and was obliged to work therefore in a reclining posture—was that of the production of the permanent records of his collections so far as he could work them up. His *Essay upon the Palms of the Amazon* is classical, but his chief publications are within the domain of the *Hepaticae*. From his earliest years this group attracted him, and his largest and most comprehensive publication is that upon the *Hepaticae* of the Amazon and Andes, now generally recognized as the most important book upon the *Hepaticae* that has appeared in recent years. The titles of Spruce's publications upon the *Hepaticae* hardly give an idea of the full extent of his work upon them and of its merit. Ostensibly descriptive and systematic, his writings are weighty in the discrimination of characters and in the adjustment of boundaries; but over and above this, they have the charm of deserving to be read between the lines, for

they abound with interjected suggestions, often most pregnant. For instance, the question of the evolution of the leafage of the Hepaticae and its relation to that of higher plants may be raised in a foot-note; the water-supply and the biological relationships of the group may be incidents of the description of the finding of a new species, and so forth. The inspiration, if not the facts, of many of the supposed new points in the biology of the Hepaticae, which their study as fashionable ancestral land-forms has recently been bringing forward, will be found by the diligent student in Spruce's writings. Had he published, as one gathers he had the intention of doing, a general treatise upon the Hepaticae, instead of dispersing his biological observations through a series of systematic papers, there is little doubt the value of his work would have been recognized earlier and more widely.

Tall and spare of frame, Spruce had, despite ill-health, a constitution of some power to endure the hardships of his many years of American life. 'I am not one of the forwardest to face perils, but once embarked I think no more of the consequences,' he says of himself, and his travels confirm it. Warm-hearted and impulsive, he was quick to wrath, yet readily appeased. A natural shyness was the frequent cause of misunderstanding between himself and others, and doubtless had, along with ill-health, to do with the comparative isolation from the botanical world of his later life. At the same time there was in him a touch of imperiousness, and whether from mortified vanity or ungratified sympathy he was inclined to resent what he regarded as his neglect by the botanical world, and showed it in a cynicism which his sense of humour at times made genial.

Mr. Slater, of Malton, and Mr. Massee, of Kew, have been so good as to furnish information regarding Dr. Spruce, and the former gentleman has also supplied the photograph of him in his later years—the only one available—from which our portrait is taken.

ISAAC BAYLEY BALFOUR.



# Studies on the Araceae.

BY

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With Plates I-III.



WHILE the Araceae have received sufficient attention from the systematists, they have not had their due from the students of histology, and especially of embryology. This seems rather surprising, considering what a peculiar and interesting Order that of the Araceae is; but, on the other hand, as only a small number of them are plants of the temperate zones, it is probable that the difficulty of procuring suitable material has had much to do with this.

It is true that Hofmeister, in his remarkable series of embryological studies, discusses and figures a considerable number of Araceae, and shows a number of peculiarities to be present, which seem to have been quite disregarded by later observers. Unfortunately, Hofmeister's accounts are very fragmentary and, although extremely suggestive, have not been followed by any others except the description of the embryo of *Pistia* by Hegelmaier<sup>1</sup>. More recently Mottier<sup>2</sup> has published an account of the early development of the ovule and embryo-sac of *Arisaema triphyllum*, but aside

<sup>1</sup> Hegelmaier, Zur Entwicklungsgeschichte monocotyledoner Keime, Bot. Zeit., 1874.

<sup>2</sup> Mottier, Bot. Gazette, 1892.

[Annals of Botany, Vol. XIV. No. LIII. March, 1900.]

from the works of these investigators I have not been able to find any references to the embryonic structures in these interesting plants.

The Araceae show many indications of being a primitive type, and this suggested a careful examination of them, in connexion with a general study of the development of the flower and embryo in the lower Monocotyledons, begun some time ago. The rich flora of Pacific America is unfortunately almost wanting in Aroids, so that except for *Lysichiton Kamtschaticense*, Schott, which is very common on the north-west coast, no material was available in California. A trip to Jamaica in the summer of 1897, however, gave me an opportunity to collect a number of forms, part of which furnished the materials for most of the present paper. Through the kindness of the Hon. W. Fawcett, Director of the public gardens, the fine collection in these was put at my disposal, and this, with the rich supplies furnished by the numerous native species, provided me with a large amount of material. Unfortunately, much of this proved to be of no value, as the flowers require great care, which it was not always possible to give, in preserving them for histological work. Moreover, at the time, the best methods of preparation were not understood, and thus much valuable material was lost.

In the summer of 1898 a trip was made to Alaska, and at Sitka abundant material of the characteristic Aroid, *Lysichiton Kamtschaticense*, was secured. At this time, the last of June, the flowers were gone, but a good supply of the young fruit in various stages of development was secured. Through the kindness of Professor Charles Hill, of Seattle, younger flowers preserved in chromic acid had been sent me, so that I was able to make a fairly complete study of this species.

On coming to England last summer, examination was made of the fine collections at Kew, and through the courtesy of the Director, Sir W. T. Thiselton-Dyer, permission was given me to avail myself of the plants growing there. I have thus been able to replace most of the spoiled material collected in

Jamaica, as well as to obtain a number of forms which I had not had an opportunity to see before. This material has not yet been worked up, but promises some interesting results.

A good deal of difficulty was experienced in the preservation of specimens for histological study. This was especially the case in some of the larger species of *Anthurium* and *Philodendron*. Most of the Araceae develop a large amount of mucilage in the floral tissues, and especially about the young seeds. In case aqueous fixing media are employed, the swelling mucilage is very troublesome, and interferes seriously with the penetration of the fixing agents. It was by the employment of these aqueous media that much of my West Indian material was made useless. So far, the best results have been obtained by using a concentrated solution of corrosive sublimate in ordinary alcohol. In some cases 10% of acetic acid was added to the solution. The specimens were embedded in paraffin, and the sections double-stained. As a general thing, the best results were obtained from a double stain of Bismarck-brown and aniline-water safranine.

The more striking results as to the structure of the ovule in *Lysichiton* have already been published<sup>1</sup>, but the details of the embryo were not discussed. The present paper is mainly concerned with the further study of this species, and also with the results of a study of two forms collected in Jamaica—*Dieffenbachia seguine* and an unnamed *Aglaonema* collected in the Hope botanical gardens near Kingston. Some preliminary notes are also given on species of *Anthurium* and *Philodendron*.

The Araceae exhibit great variety in their flowers, which have been very thoroughly studied, especially by Schott<sup>2</sup> and Engler<sup>3</sup>. The flowers may be hermaphrodite and all similar,

<sup>1</sup> Campbell, Bot. Gazette, March, 1899.

<sup>2</sup> Icones Aroidearum, Vienna, 1857. Schott: Genera Aroidearum, Vienna, 1858.

<sup>3</sup> Engler: Vergleichende Untersuchungen über die morphologischen Verhältnisse



as in *Anthurium* and *Lysichiton*, or more commonly they are unisexual, in which case they may be more or less intermingled, e.g. *Spathicarpa*, or more frequently with the female flowers at the base of the spadix and the male occupying the upper portion. Dioecism is rare, but may occur in a few forms, e.g. *Arisaema triphyllum*.

#### DIEFFENBACHIA SEGUINE, Schott.

*Dieffenbachia seguine* is one of the commonest of the native West Indian Araceae, and a good supply of the early stages of the flower was secured, but unfortunately it was too early to procure the developing fruits, so that for the present the development of the embryo must remain unstudied.

Owing to the delicate character of the tissues of the young flowers they are easily sectioned, and their development can be readily followed. The large spadix is enclosed in the rolled-up spathe, and is characterized by having the basal part occupied by the female flowers, completely adherent to the base of the spathe, so that they are developed on one side only of the spadix. Each pistillate flower is composed of two nearly separate carpels, each containing a single large basal ovule. Scattered between the carpels are white, club-shaped staminodia. No trace of a perianth is to be seen in either male or female flowers. The upper part of the spadix projects partly out of the spathe, and is completely covered with the crowded male flowers, each of which consists of a stalked synangium, recalling in form the peltate sporophylls of *Equisetum*. As is usually the case among Aroids, the inflorescence is decidedly proterogynous.

In sections through the base of a very young spadix, the young female flower appears as a hemispherical protuberance with a carpel arising on either side (Fig. 1). The staminodia (*st.*) are already very evident. The central prominence may be considered as the apex of a lateral shoot bearing the two carpellary leaves, one of which may perhaps develop

a little before the other, but this point was not certainly determined.

As the flower grows, the central portion projects strongly, and may be called the placenta, as the ovules arise from it as lateral outgrowths in the axils of the two carpellary leaves (Fig. 3). The ovules must be regarded as axial structures in *Dieffenbachia*, which thus agrees with most other low Monocotyledons which have been investigated. Indeed, there is constantly increasing evidence to show that the single axial ovule is the primitive form.

The carpels grow up rapidly about the ovule, and arch over toward the placenta (Fig. 3). They become very thick, so that the ovarian cavity is small, and a narrow canal is left between the thick margin of the carpel and the central columnar placenta. Later, the upper surface of each carpel develops a large button-shaped stigma. The carpels are coherent at their lateral edges, but there is a deep suture, and the two stigmas are quite separate.

The young ovule projects obliquely from the base of the placenta, and very early a vascular bundle becomes evident, traversing the short thick funiculus, and joining a bundle in the placenta. The ovule at this time is nearly straight. The two integuments are indicated and the archesporial cell can be distinguished. Very soon the growth of the upper side is accelerated, and the ovule assumes the anatropous condition found in the later stages.

#### *The Male Flowers.*

The male flowers in *Dieffenbachia* completely cover the upper part of the spadix. Each consists of a synandrium made up of a varying number, usually four or five, of coherent stamens borne upon a peltate receptacle. In cross-sections of the young spadix, the young synandrium appears as a mushroom-shaped elevation (Fig. 16) strikingly resembling a corresponding stage in the development of the sporophyll in *Equisetum*. About the margin and projecting

downward are the young anthers (Fig. 16 *sp.*). Each anther develops two loculi.

Owing to the uniformity in size and contents of the cells composing the young stamens, it is not possible to determine with certainty the exact origin of the sporogenous cells. The shaded cells in the figure (Fig. 17) probably represent the beginning of the archesporial tissue, but it is quite possible that some of the surrounding cells may also contribute to this. Outside of the sporogenous tissue there are about four layers of cells which form the wall of the anther.

The further history of the anther was not followed in detail. The young pollen-spores, which are of the bilateral type, are oval in form (Fig. 18) and surrounded by a firm membrane. The cytoplasm fills the cell, and the nucleus is almost central in position. The young spores are embedded in a thick layer of nucleated protoplasm, doubtless derived from the broken-down tapetum, and perhaps in part from a portion of the sporogenous cells. This point was not, however, investigated.

As the spores ripen they increase a good deal in bulk and become almost globular in shape. Vacuoles appear in the cytoplasm, and the nucleus divides into two—a large vegetative, and a smaller generative nucleus, the latter enclosed in a small lenticular cell (Fig. 19). No cases were seen where the generative nucleus had divided, and this final division does not probably occur until after the germination of the pollen-spore, which I had no opportunity of studying.

#### *The Ovule.*

The ovule, as we have seen, is an axial structure, and not an outgrowth of the carpel. In the earliest stage seen (Fig. 4), the ovule is nearly straight with a massive base and the beginning of the first integument. The nucellus is nearly hemispherical in form, and the single hypodermal archesporial cell is already evident. Very soon the second integument makes its appearance below the first one, which reaches nearly to the apex of the nucellus; the latter has also elongated. The accelerated growth of the cells on the upper side



of the funiculus has begun now to push the nucellus downward, and the ovule begins to show the anatropous form found at maturity.

The primary archesporial cell undergoes a transverse division resulting, in all the cases seen, in two cells of unequal size, of which the upper one seems to give rise at once to the embryo-sac, while the lower one may either divide once (Fig. 6), or probably in some cases remains undivided. No tapetal cells were seen in these young stages, although in the older stages a cell was sometimes seen above the embryo-sac which may possibly have been a tapetal cell (Fig. 8, *i*).

The further history of the embryo-sac offers nothing strikingly different from that of the typical Angiosperms. The embryo-sac mother-cell increases in size, and finally destroys the smaller sister-cell (or cells) below it. The nucleus is large and conspicuous, and after some further enlargement of the embryo-sac, which now begins to crowd upon the smaller tissue, it undergoes the usual division into two, one of which moves to each end of the sac (Fig. 8). The further division of these then follows in the usual manner.

With the rapid enlargement of the embryo-sac the lateral cells of the nucellus, which at first form two or three layers (Fig. 6), become very much compressed, and the nuclei and other contents show signs of disintegration. Even before the nuclear divisions in the young sac are completed, the lateral tissues of the nucellus are almost completely destroyed, and the embryo-sac is apparently in close contact with the inner integument. The apex of the nucellus, however, persists. This at first consists of two layers of cells (Figs. 5, 6), but later these may divide by periclinal walls so that there are three layers (Figs. 8, 9). The cells of the apex of the nucellus become much extended vertically and form a conspicuous cap above the apex of the embryo-sac.

There is but little difference in the appearance of the nuclei at the upper and lower ends of the sac. They all stain readily and show a small but distinct nucleolus. The arrangement of the cells forming the young egg-apparatus and the

antipodal group is very similar (Fig. 10), but later they show some differences. So far as could be told from a somewhat imperfect series of stages, the fusion of the polar nuclei takes place some time before fertilization—at any rate in all cases where the structures of the embryo-sac were complete, the polar nuclei had entirely fused.

The mature ovule (Fig. 9) has a very short and thick funiculus, and has assumed the anatropous form. The nucellus is relatively very small, and except at the apex and base has had its cells completely destroyed by the growth of the embryo-sac. The inner integument is only moderately developed, but the outer one is very thick, especially on the side next the funiculus.

The cells of the egg-apparatus (Figs. 13, 14) are much alike. In the specimen figured there was present in the egg-cell, besides the egg-nucleus, a small deeply-stained body (*g.*), apparently a nucleus, which may have been one of the male nuclei; but the pollen-tube could not be detected, and both of the synergidae were intact. The antipodal cells (Figs. 10, 12) are large and conspicuous, and closely resemble the cells of the egg-apparatus. A peculiar character of this Aroid, which however seems not uncommon in the family, is the presence of starch-granules in the unfertilized embryo-sac, especially about the endosperm-nucleus (Figs. 11, 15).

Unfortunately, although repeated efforts were made, I was unable to procure specimens in which the ovules were fertilized, so that I am unable to state what is the further history of the embryo-sac and embryo.

#### AGLAONEMA.

The genus *Aglaonema* belongs to the East Indian and Malayan region, but specimens were flowering freely in the Hope botanical garden near Kingston. The plants were labelled *Dieffenbachia Aglaonema*, but were determined to be *Aglaonema*—probably *A. commutatum*, Schott. The genus differs from *Dieffenbachia* in having the carpels entirely separate, and the base of the spadix quite free from the



spathe. While I was unable to procure a complete series of stages, still a considerable number were secured, especially of the later stages which were missed in *Dieffenbachia*.

The solitary carpels are tipped with the very large slightly funnel-shaped stigma, and the large solitary ovule arises from the base of the ovary. A section of the stigma shows it to be composed of very closely set elongated papillae, which become shorter toward the centre of the funnel. There is a short but evident canal extending to the ovarian cavity, but it is completely filled with the stigmatic papillae.

The ovule, like that of *Dieffenbachia*, is very massive, but the integuments are not so largely developed (Figs. 22, 27). To judge from the very small number of early stages obtained, the general development is much like that of *Dieffenbachia*. All the nuclei are extraordinarily large, and the plant would probably prove a good one for cytological study. The embryo-sac, as in *Dieffenbachia*, soon destroys the lateral tissue of the nucellus, but leaves the apical part as a cap at the top of the embryo-sac. At the time of fertilization the free portion of the outer integument is very slightly developed, and the opening of the micropyle is formed by the inner integument alone. After the endosperm begins to develop, the outer integument grows until its margin is on a level with that of the inner one, but does not extend beyond it (Fig. 27). On the side next the funiculus, the outer integument is scarcely developed at all, and it is difficult to say just how much of the base of the ovule is to be regarded as nucellus, and how much as funiculus.

Most of the specimens examined had already begun to form the endosperm, and no satisfactory preparations of the egg-apparatus or antipodal cells were secured. One puzzling case was encountered, shown in Figs. 22-24. The embryo-sac here was of full size, and at the base was a very sharply defined mass of densely granular cytoplasm containing two large nuclei, which were apparently in the prophases of division. At the apex of the sac was a rounded mass of protoplasm with a single nucleus, apparently the egg, and in

the neighbouring sections the synergidae could be distinguished (Fig. 23). The endosperm-nucleus could not be found, nor was there any trace of a third antipodal cell. Whether this peculiar condition of things is normal in *Aglaonema* remains to be seen, as no other specimens were found in which the egg-apparatus or early stages of the antipodal cells could be made out.

### *The Endosperm.*

Very soon after fertilization has been effected the primary endosperm-nucleus divides. Whether the first division is accompanied by the formation of a division-wall could not be determined; but it is not impossible that such is the case, as Hofmeister described such a division for *Pothos longifolia*. While the number is still very small (Fig. 26), walls have been formed between the large nuclei, and the embryo-sac is thus filled with the endosperm, almost from the beginning. This early filling up of the embryo-sac is very characteristic of the Araceae, and Hofmeister called attention to it in a number of genera, although his work seems to have been largely neglected in recent studies on the embryo-sac. There is not found in these plants—at least in the forms hitherto examined—the development of a peripheral protoplasmic layer in which are embedded the free nuclei.

The endosperm-nuclei are very large and stain readily. They possess one large nucleolus (Fig. 25) or sometimes two or more. They are separated by delicate cell-walls, which are, however, perfectly apparent. The endosperm-cells are usually largest at the apex of the embryo-sac, and smallest at the antipodal end. They contain little granular contents.

The embryo-sac is at first broadly oval in outline (Figs. 22, 26), but later elongates a good deal with the increase in size of the ovule, and there is a marked bend produced at the base of the sac, by its extension on the side next the funiculus. This bending of the embryo-sac is a very constant feature in the species under consideration (Figs. 27, 31).

*The Antipodal Cells.*

There can usually be detected a group of cells at the base of the endosperm, which from their position and general appearance are probably the antipodal cells. Whether there are originally three of these, as in other similar cases, remains to be seen. In the older stages (Fig. 30), they form, usually, a nearly hemispherical mass of cells, with large nuclei, and sometimes, at least, with denser contents than the adjacent cells of the endosperm. Just how many of these there may be was not positively determined, but the number is probably not less than a dozen. As a rule this group of cells is situated at the original base of the sac, and not in the prolongation. Fig. 32 shows a rather remarkable group of cells which was observed in one instance occupying the lower extremity of the sac. This cell-mass looked almost like an embryo, but was probably only an abnormally developed mass of antipodal cells.

In general appearance, this group of antipodal cells is quite like that of *Lysichiton*, which, as the writer<sup>1</sup> showed, possesses an increased number of antipodals derived from subsequent division of the original three. This mass is also not unlike the group of antipodals in *Sparganium* and many Grasses, and adds one more to the increasing number of Angiosperms in which the number of antipodals normally exceeds the three typical of most forms.

A number of young embryos were found, but these will be considered later on. In a good many cases, although careful search was made, no embryo could be discovered, and the same thing has been found in species of *Anthurium*. What the meaning of this is, must remain for the present unanswered. It is possible that the endosperm is capable of developing without fertilization, or it may be that for some reason the embryo fails to develop, although fertilization is effected. I hope to be able to settle this question later, as I have collected

<sup>1</sup> Campbell, loc. cit.



at Kew an extensive series of ovules of *A. commutatum*, which it is hoped will show the missing stages.

As the embryo-sac develops, the surrounding tissues of the ovule, especially the base and the funiculus, increase very much in thickness, so that the embryo-sac occupies only a relatively small portion of the whole ovule. This great development of the outer ovular tissues suggests somewhat the perisperm-form in the Scitamineae and Piperaceae.

#### ANTHURIUM.

A number of species of *Anthurium*, including the common native *A. cordifolium*, Kth., and a larger stemless species (*A. Huegelii*), as well as several others, were collected in Jamaica, but it was found extremely difficult to prepare the specimens satisfactorily, and in consequence very little of the material proved of any value, and the results obtained were very fragmentary. In a few instances sections of the ovule, before fertilization, were obtained, and so far as could be determined, the ovule at this stage is of the usual type, with the egg-apparatus and other structures entirely normal. The ovule is much more slender than in *Dieffenbachia* or *Aglaonema*, and there are often two ovules in each division of the compound ovary, which is composed of two entirely coherent carpels. The flowers in *Anthurium* are all similar, and are densely crowded on the spadix, which they completely cover. As in other Araceae, the flowers are markedly proterogynous, so that self-pollination is impossible. In many species examined it was impossible to find any fruitful spadices, although the ovary often enlarges, apparently without being fertilized. At least these young fruits are frequently encountered with the ovules entirely abortive.

In my recent collections of these plants, one species, *A. violaceum*, var. *leucocarpum*, was found, which fruits perfectly. I was informed by the gardener at Kew, that this species was much infested by a small ant, and I frequently saw these insects running about over the plant, and probably serving to

convey the pollen from the older flowers to the receptive stigmas of the younger ones. Whether ants are the usual agents of pollination in this genus, I do not know.

The endosperm, in all the species of *Anthurium* examined, very soon fills up the embryo-sac with a solid, large-celled tissue, very much as in *Aglaonema* (Figs. 53, 54). Here also, difficulty was experienced in making out the embryo, and it seems questionable whether an embryo is present in many cases; but unfortunately the necessary stages could not be secured, and the question must remain open for the present.

In most species of *Anthurium*, as in most of the Aroids examined, there is a large development of mucilage about the ovule, and this interferes very much with the proper fixing of the material for histological study. Watery fixing fluids swell up the mucilage so strongly as to interfere with the proper penetration of the fixing fluid, and one must have recourse to alcoholic solutions.

#### PHILODENDRON.

Material was collected in Jamaica of *Philodendron lacerum*, Schott, and *P. tripartitum*, Schott, but neither proved very satisfactory for study, and very little was done with them. The ovules are small, and borne upon a slender funiculus (Fig. 56), which is provided with secretory papillate hairs. I was unsuccessful in procuring the older stages, and cannot state how they compare with those of other genera studied.

Hofmeister figures the ovules of *P. Imbe*, but does not show the character of the endosperm.

#### LYSICHITON.

*Lysichiton Kamtschaticense*, Schott, is a remarkable Aroid which is very common in the coast region of North Pacific Asia, and North America, extending as far south as Northern California. It is an exceedingly common and conspicuous plant about Puget Sound, and along the coast of Alaska, where I collected it in June and July, 1898. The plant grows

in low wet ground, and closely resembles in general appearance *Anthurium acaule*. Indeed both in the leaves and flowers it seems to be nearer to *Anthurium* than to *Symplocarpus*, with which it is usually associated. It was late for the flowers at the time of my visit, and most of my material showed only the later stages of the embryo-sac and the embryos. For the earlier stages, I had to depend on the material furnished me by Professor Hill, of Seattle.

The flowers, like those of *Anthurium*, are hermaphrodite, and like many species of *Anthurium*, the ovary is two-celled, with two ovules in each cell. The ovules themselves, however, are much larger and more massive, and are orthotropous. They are attached to the inner wall of the ovarian cavity, near the base, but none of the material was young enough to determine positively whether they arose from the axis, as in *Dieffenbachia*, or from the carpel itself. To judge from a comparison with the older carpels of *Dieffenbachia*, it is probable that in *Lysichiton* also, the ovules are outgrowths of an axial placenta.

The young ovule (Fig. 34) has a very broad and short funiculus. As in *Dieffenbachia* and *Aglaonema*, the nucellus is very small. As in these forms, the micropyle is formed by the inner integument alone, the outer integument being very short. The latter is, however, extraordinarily massive, so that the breadth of the ovule considerably exceeds its height, giving it a most characteristic form. At this time, an examination of the epidermal cells of the base of its outer integument shows that they have begun to enlarge, and their contents become dense and the nuclei large. Later, these cells grow out into elongated papillae, and the base of the ovule is surrounded by a thick ring of these secretory hairs which develop a mass of transparent mucilage in which the ovule is completely embedded, and which make it very difficult to preserve the older ovules without shrinkage; even taking all the precautions I could, much of my material proved of little use.

The earliest stages procurable had the parts of the embryo-sac already complete, and the young embryo-sac showed the egg-apparatus of the ordinary form, and the three antipodal



cells at the lower end. The two polar nuclei were present, and quite separate. The nucellus, while small compared to the size of the ovule, is larger relatively than in the other forms which we have considered. It is oval in form, and the lateral walls are composed of three or four layers of cells, while at the apex there are five or six layers of cells above the embryo-sac.

In the youngest stages encountered (Fig. 35) the nuclei in the embryo-sac were much alike, of moderate size, and showed a large nucellus. There could be detected above the apex of the sac in some cases, what looked like the remains of tapetal cells, but whether such cells are always formed cannot now be stated.

As the embryo-sac grows, it becomes somewhat longer (Fig. 36), but unlike that of most Araceae, it does not destroy the nucellar tissue completely, the lateral walls of the nucellus being perfectly evident for a long time after the embryo has begun to develop (Fig. 43).

With the increase in the size of the embryo-sac, the egg-apparatus assumes its fully developed form (Figs. 37, 38). The egg-cell, as usual, is somewhat larger than the synergidae, and projects below them. It contains little granular cytoplasm, and the nucleus, with its very large nucleolus, lies near the free end, close to the wall. The synergidae have much more densely granular contents, and the nuclei do not differ appreciably from the nucleus of the egg.

The polar nuclei are of about the same size as the nuclei of the egg-apparatus, and probably remain separate until after the fecundation of the egg-cell. At any rate, in most of the cases examined, they were quite separate, although often in close contact (Fig. 41). They usually stain more uniformly than the other nuclei, but otherwise show no peculiarities.

#### *The Antipodal Cells.*

The antipodal cells in *Lysichiton* are very peculiar in their behaviour. At first they resemble the ordinary form, and their nuclei resemble closely in size and appearance those of the egg-apparatus. Soon, however, a difference is manifest.

The antipodal cells increase considerably in size (Fig. 40), and the contents become denser. At the same time there is a marked increase in the size of the nuclei, which show a coarsely granular appearance, and stain strongly. Sometimes they exhibit an appearance indicating that they are about to divide, but no cases were seen where they had divided in the unfertilized sac. Later, as we shall see, there is a division of these cells, similar to what was observed in *Aglaonema*. Hofmeister describes and figures a great increase in the size of the antipodal cells in *Arum orientale*, but gives no account of their nuclei, nor does he seem to have found any cases where the antipodal cells subsequently divided.

#### *The Endosperm.*

The formation of a solid endosperm is also characteristic of *Lysichiton*, but unfortunately, here also, the earliest stages were missing. The embryo-sac soon becomes filled with a solid tissue, whose cells are rather smaller, and have denser cytoplasm at the basal part than at the apex. The cells surrounding the embryo (Fig. 44) are larger, and the granular cytoplasm is mainly restricted to the neighbourhood of the nucleus.

While the endosperm has been forming, there has been a marked growth of the integuments. The outer integument now extends beyond the inner one (Fig. 43), and the base of the latter has thickened a good deal. The embryo-sac is still separated from the inner integument by several layers of nucellar tissue, and the base of the ovule is very much thickened, so that, as in *Aglaonema*, the embryo-sac constitutes only a small part of the bulk of the ovule.

While the endosperm has been forming, the antipodal cells also increase very much in bulk, and undergo division, so that they form a large and conspicuous mass of tissue at the base of the embryo-sac. Sometimes they are very sharply separated from the endosperm-cells above them (Fig. 45), but this is not always the case, and not infrequently it is not easy



to decide just how many of the basal cells really belong to the antipodal group. In the specimen figured, the line of junction between the two is very evident. In this case there were eight antipodal cells, which were characterized by very large nuclei and granular cytoplasm. The nuclei showed some signs of degeneration, especially in the upper cells, and it is doubtful whether they would divide any further. In other cases, the antipodal cells were somewhat smaller and more numerous, but being less clearly distinguishable from the lower endosperm cells, it was not possible to be sure exactly how many of them there may be, but there are probably a dozen or more in some cases.

#### *The Embryo.*

The earliest stages of the embryo were not found in *Lysichiton*, but in *Aglaonema* several very young embryos were met with, which correspond closely with those of *Pothos* figured by Hofmeister. The earliest stage encountered was a two-celled embryo, shown in Fig. 28. The two cells were of nearly equal size. Of these the terminal one, i. e. the one at the free end, undergoes a transverse division, resulting in a three-celled stage like that generally met with in Monocotyledons. It is the terminal cell, which probably gives rise to the greater part of the embryo, although in the three-celled stage shown the terminal cell is the smallest of the three. As this was the only specimen met with in this condition, we cannot say whether this is normal. In a somewhat older one (Fig. 33), the terminal segment was very much larger, and had already undergone division. Here the end of the embryo was decidedly pointed, but this is probably not a constant character. The first division in the terminal segment in this case was somewhat oblique, and followed in the larger cell by a wall meeting the first one nearly at right angles. The appearance of this embryo was very much like that of certain grasses figured by Nörner<sup>1</sup>. The stages following were not

<sup>1</sup> Nörner, Flora, 1881.

found in *Aglaonema*, but somewhat older ones were obtained in *Lysichiton*.

The youngest embryo of *Lysichiton* which was found is shown in Fig. 33. The broad base of the embryo in this case, and the absence of the primary transverse walls, do not agree with the older stages, of which many were found, but resemble Hegelmaier's figures of *Pistia*<sup>1</sup>, and also Hofmeister's figure of the young embryo of *Calla palustris*.

Stages like the one shown in Fig. 44 were frequently encountered. Here the transverse segmentation of the basal region is still very evident; and probably the early divisions were like those in *Aglaonema*, and most Monocotyledons which have been investigated. There is, as yet, no differentiation of the primary organs of the embryo, which takes place very late in these plants. No suspensor is developed, nor is there any enlargement of the basal cell, which is so marked in many Monocotyledons—e. g. *Alisma*, *Naias*, *Lilaea*, &c. In the absence of the suspensor, and the form of the embryo itself, these Aroids recall the Grasses, and also the embryo of *Sparganium*. The absence of a suspensor is clearly correlated with the complete investment of the young embryo by the endosperm, with which all of its superficial cells are in close contact, and from the cells of which they can abstract the substances necessary for the growth of the embryo.

As the embryo enlarges it becomes somewhat flattened in the plane of the cotyledon. The first indication of external differentiation is the formation of a depression near the base, in the middle line of the cotyledon (Figs. 47, 48). This is the beginning of the stem-apex, but it is quite impossible to trace it back to the early segments of the embryo, which have become quite unrecognizable by this time. Probably the whole cotyledon (which comprises the greater part of the embryo), and the stem as well, are products of the terminal one of the three primary segments, but it is not possible to prove this positively. At this time the embryo is composed

<sup>1</sup> Hegelmaier, loc. cit.

of apparently homogeneous tissue, and it is not until a late period that the vascular bundles are evident.

As the embryo enlarges it becomes somewhat heart-shaped (Fig. 50 *a, b*), and near the base of the cotyledon there can be seen for a short distance a strand of procambium. The stem-cleft has become deeper, but is almost completely enclosed by the base of the cotyledon which forms a sheath about it. Up to this time, no certain evidence of the primary root can be traced, and it is evidently very late in making its appearance.

Fig. 52 shows a median section, at right angles to the plane of the cotyledon, of a nearly full-grown embryo. The young vascular bundles could be seen, although they were very slightly developed. Within the cleft at the base of the cotyledon is the protuberance which probably represented not only the stem-apex, but also the beginning of the second leaf. A short branch of the bundle of the cotyledon runs into this stem-rudiment (or leaf?), and a larger branch is continued obliquely into the rudiment of the root (*r.*), which is very evidently of lateral origin, and shows no very clear arrangement of the tissues.

Fig. 55 shows two sections of a full-grown embryo of *Anthurium cordifolium*, which in the main agrees with that of *Lysichiton*, but the stem-apex is higher up, and the root, which is much better developed, is terminal, as it is in most investigated Monocotyledons.

It is evident that, among the Araceae, the development of the embryo shows a good deal of variation, and more information regarding the early divisions of the embryo is much needed. If the early stages shown in *Aglaonema* are normal, there are two well-marked types at least—that where the embryo first becomes divided into two transverse segments, and that in which there is a more or less regular quadrant-formation, as in *Pistia*, and in the very small number of young embryos found in *Lysichiton*. Most of the older embryos seen in the latter, however, point to an early segmentation like that in *Aglaonema*.



In *Pistia*, which so far is the only Aroid whose embryology has been studied at all completely, formation of quadrants in the young embryo is associated with a lateral formation of the root like that in *Lysichiton*, and is very suggestive of the formation of the root in *Isoëtes*; indeed the early segmentation of the embryo, as well as the later structure, is very much alike in *Pistia* and *Isoëtes*.

#### SUMMARY AND CONCLUSION.

1. In *Dieffenbachia* and *Aglaonema* there is no question as to the axial origin of the ovule; this is probably the case also in *Lysichiton*, but not so certainly in *Anthurium*.

2. In all of the forms examined there is a large development of the base of the ovule, and in *Aglaonema* and *Lysichiton* of the integuments also, so that the embryo-sac does not occupy as much of the ovule as is usually the case.

3. Up to the time of fertilization, the embryo-sac in the forms examined (except possibly *Aglaonema*) shows the ordinary type of development.

4. The endosperm in all the forms examined very early forms a continuous tissue, completely filling the embryo-sac.

5. The antipodal-cells in *Lysichiton* become very large, and undergo secondary divisions, so that a large group of cells results; in *Aglaonema* a similar group of cells was seen, but its development could not be traced; the antipodal-cells could not be certainly distinguished in the older embryo-sacs of *Anthurium*.

6. There are probably two types of segmentation of the young embryo; in the first there are formed two transverse divisions before any longitudinal walls appear; in the second there is a quadrant-formation, suggestive of the early divisions in the Fern-embryo; the differentiation of the organs takes place at a late period, and it is not possible to trace them back with certainty to the primary divisions of the embryo.

7. The cotyledon is very large, the stem and root much

less conspicuous; the root in *Lysichiton* seems to be of lateral origin, as it is in *Pistia*.

8. A suspensor is never developed, this being associated with the complete investment of the embryo by the endosperm.

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Owing to the small number of types of Araceae which have been investigated, and the incomplete knowledge of most of those which have been studied, it is not yet possible to be very certain as to the conclusions to be drawn from these studies.

Engler<sup>1</sup> considers the forms like *Pothos* and *Anthurium*, with hermaphrodite flowers, to be the primitive forms of which those with unisexual flowers are reduced forms. It seems quite as likely that the reverse is the case, especially as *Anthurium* and its allies have more complex pistils than most of the unisexual forms. Moreover, from comparison with other low Monocotyledons, it seems probable that the forms with a single carpel and single basal ovule are the most primitive. However, we are not in a position yet to decide this important question.

The development of the embryo-sac requires much more attention. In *Arisaema triphyllum* there are regularly four archesporial cells resulting from a longitudinal division of the primary archesporial cell<sup>2</sup>, and it remains to be seen whether this may not be the case in other forms, instead of the simpler type found in *Dieffenbachia*.

The early development of a solid endosperm seems to be a pretty constant character in all the forms yet examined, and is an important one. A comparison with the prothallial tissue of *Isoetes* or *Selaginella* is inevitable, and it probably represents a primitive condition as compared with most Angiosperms.

The great development of the antipodal cells is also to be considered as an evidence of the primitive nature of the

<sup>1</sup> Engler and Prantl, *Natürliche Pflanzenfamilien*.

<sup>2</sup> Mottier, l. c.

Araceae. This condition is now known in a number of other low Monocotyledons, especially the Grasses, and is also a regular phenomenon in *Sparganium*. It is highly probable that further research will reveal a similar condition in other low Monocotyledons.

The embryo resembles most nearly that of the Grasses and *Sparganium*, like these being quite destitute of a suspensor, and having the cotyledon largely developed. However, as the absence of the suspensor is obviously associated with the early development of the endosperm, it may be that this does not imply a relationship. If, however, these lower Monocotyledons have been derived from Fern-like ancestors similar to *Isoëtes*, we should not expect to find a suspensor developed in them. The lateral development of the primary root, also, in such forms as *Lysichiton* and *Pistia*, is suggestive of the embryo of *Isoëtes*.

In connexion with this preliminary study of the Araceae, which it is hoped may soon be materially added to, attention was called, while collecting in Jamaica, to certain remarkable similarities in habit between them and the Piperaceae. Both in the form of the leaves and inflorescence, as well as in certain anatomical characters, e.g. the distribution of the vascular bundles in the stem of *Peperomia*, there are very curious parallelisms. The structure of the flower and position of the ovule are also similar. That these really indicate any relationship between the two orders, it would be rash to assume; but the possibility of such a connexion may very well be considered. So far as I am aware, no careful embryological study has been made of the Piperaceae, and it will be necessary to do this before it is safe to draw conclusions.



## EXPLANATION OF FIGURES IN PLATES I-III.

Illustrating Professor Campbell's paper on the Araceae.

## PLATE I.

Plate I, Fig. 21, refers to *Aglaonema* sp., the others to *Dieffenbachia seguine*, Schott.

Fig. 1. Longitudinal section of a young female flower of *Dieffenbachia*, showing the apex (x) of the floral axis; the carpellary leaves, *car.*, and staminodia, *st.* x about 45.

Fig. 2. The same, more highly magnified.

Fig. 3. Median section through the pistil of an older flower; *pl.* placenta, *ov.* ovule. x about 40.

Fig. 4. Median section of a young ovule, showing the first integument *in*<sup>1</sup>, and the primary archesporial cell. Leitz, oc. 1, obj. 7.

Fig. 5. A somewhat older ovule; the second integument is beginning to develop, and the archesporial cell has divided.

Fig. 6. Apex of an ovule with the archesporium divided into three cells; the upper larger one becomes the embryo-sac.

Fig. 7. The nucellus of an older ovule; the embryo-sac has elongated, and the nucleus is preparing for division.

Fig. 8. Embryo-sac with two nuclei. Leitz, oc. 1, obj. 7.

Fig. 9. Median section of a mature ovule. *ma.*, the embryo-sac; *nu.*, apex of nucellus; *in*<sup>1</sup>, *in*<sup>2</sup>, the integuments.

Fig. 10. Two longitudinal sections of an embryo-sac after the differentiation of the egg-apparatus and antipodal cells.

Fig. 11. One of the antipodal cells and the endosperm-nucleus of a somewhat older sac; the endosperm-nucleus is surrounded by starch-granules.

Fig. 12. The antipodal cells from a slightly older sac; they have collapsed somewhat in the process of embedding.

Fig. 13. The upper part of the nucellus and embryo-sac from a mature ovule, showing the two synergidae. Leitz, oc. 1, obj. 7.

Fig. 14. The egg from the same embryo-sac; the small dark body, *g.*, is perhaps one of the male nuclei from the pollen-tube.

Fig. 15. Base of the embryo-sac, showing one of the antipodal cells, together with the endosperm-nucleus and the surrounding starch-granules.

Fig. 16. Median section of a very young synandrium, showing two young pollen-sacs, *sp.* x about 45.

Fig. 17. A single pollen-sac more highly magnified; the shaded cells represent the probable extent of the archesporium. Leitz, oc. 1, obj. 7.

Fig. 18. Section through an older pollen-sac, showing the young pollen-spores embedded in the nucleated protoplasm derived from the disintegration of the tapetum. Leitz, oc. 1, obj. 7.

Fig. 19. Section of a nearly ripe pollen-spore showing the generative cell, *g*., and the vegetative nucleus, *v*. Leitz, oc. 1, obj. 7.

Fig. 20. Wall of the ripe pollen-sac.

Fig. 21. *a*, Young embryo-sac of *Aglaonema* sp., with four nuclei, two only of which appear in this section; *b*, one of the nuclei from the base of the sac. Leitz, oc. 1, obj. 7.

#### PLATE II.

Figs. 22–32 refer to *Aglaonema* sp., the others to *Lysichiton Kamtschaticense*, Schott.

Fig. 22. Nearly median section of an ovule of *Aglaonema*; two antipodal cells, *ant*., only could be distinguished. Leitz, oc. 1, obj. 3.

Fig. 23. *a*, the egg; *b*, the synergidae from the same. Leitz, oc. 1, obj. 7. No polar nuclei could be detected.

Fig. 24. The two antipodal nuclei from the same embryo-sac; these are in the prophase of division.

Fig. 25. Upper end of the nucellus and embryo-sac with the endosperm already developed. No embryo was visible.

Fig. 26. Nearly median section of an ovule shortly after fertilization; the embryo-sac is already filled with a large-celled endosperm, and the embryo is three-celled. Leitz, oc. 1, obj. 3.

Fig. 27. An older ovule, with the embryo-sac and embryo, *em*.

Fig. 28. Two-celled embryo. Leitz, oc. 1, obj. 7.

Fig. 29. Three-celled embryo.

Fig. 30. Group of antipodal (?) cells from an older embryo-sac.

Fig. 31. Longitudinal section of the embryo-sac from an older ovule, showing its bent form.

Fig. 32. Peculiar group of antipodal (?) cells from an older embryo-sac.

Fig. 33. Median longitudinal section of a young embryo of *Lysichiton*.

Fig. 34. Longitudinal section of a young ovule of *Lysichiton*; *ma*., the embryo-sac. Leitz, oc. 1, obj. 3.

Fig. 35. Nucellus and young embryo-sac of *Lysichiton*. Leitz, oc. 1, obj. 7. *t*., tapetal (?) cells above the embryo-sac.

Fig. 36. Nucellus and embryo-sac from an older ovule.

Fig. 37. Nearly mature embryo-sac in longitudinal section, showing part of the egg-apparatus and one of the antipodal cells. Leitz, oc. 3, obj. 7. *sy*., a synergid; *o*., the egg.

Fig. 38. The egg and one of the synergidae from a mature embryo-sac.

Fig. 39. Transverse section of the antipodal cells from a mature embryo-sac. Leitz, oc. 3, obj. 7.

Fig. 40. Two of the antipodal cells from the same sac; the nucleolus of the lower one has been displaced in sectioning.

Fig. 41. The two polar nuclei in process of fusion. Leitz, oc. 3, obj. 7.

Fig. 42. Two longitudinal sections of a young embryo of *Lysichiton*.



## PLATE III.

Figs. 43-52 refer to *Lysichiton*; 53, 54, 55, *Anthurium*; 56, *Philodendron*.

Fig. 43. Median section of the fertilized ovule of *Lysichiton*.  $\times$  about 40. *nu.*, apex of nucellus; *em.*, embryo; *end.*, endosperm; *ant.*, antipodal cells.

Fig. 44. Longitudinal section of the embryo embedded in the large-celled endosperm. Leitz, oc. 1, obj. 7.

Fig. 45. Antipodal cells from an ovule of about the same age as the one shown in Fig. 43. Leitz, oc. 1, obj. 7.

Fig. 46. Median longitudinal section of a young embryo. Leitz, oc. 1, obj. 7.

Fig. 47. Outline of an older embryo, showing the beginning of the stem, *st.*, and the cotyledon, *cot.* Leitz, oc. 1, obj. 3.

Fig. 48. Two longitudinal sections of an older embryo. Leitz, oc. 3, obj. 3.

Fig. 49. The stem-region of the same embryo more highly magnified.

Fig. 50. Three longitudinal sections of a still older embryo. Leitz, oc. 1, obj. 3.

Fig. 51. Transverse section through the stem region of an older embryo.  $\times$  about 25.

Fig. 52. Longitudinal median section of a nearly mature embryo, showing the lateral position of the primary root, *r.*  $\times$  about 25.

Fig. 53. Longitudinal section of an ovule of *Anthurium Huegelii* (?), showing the large-celled endosperm. No embryo could be detected. Leitz, oc. 1, obj. 3.

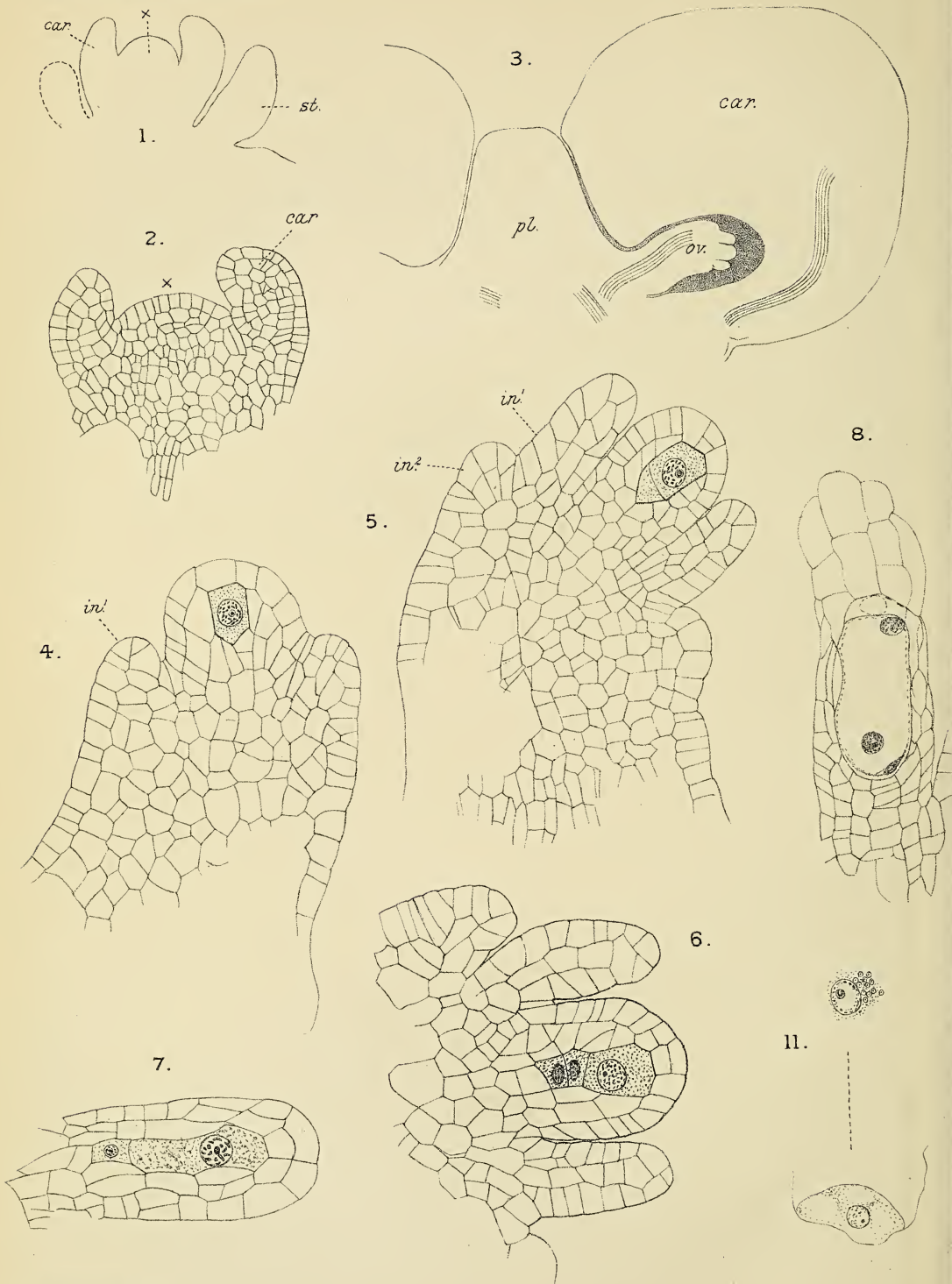
Fig. 54. Ovule of *Anthurium cordifolium*, Kth., showing the large-celled endosperm. No embryo was evident. Leitz, oc. 1, obj. 3.

Fig. 55. Two longitudinal sections of a full-grown embryo of *Anthurium cordifolium*.  $\times$  10.

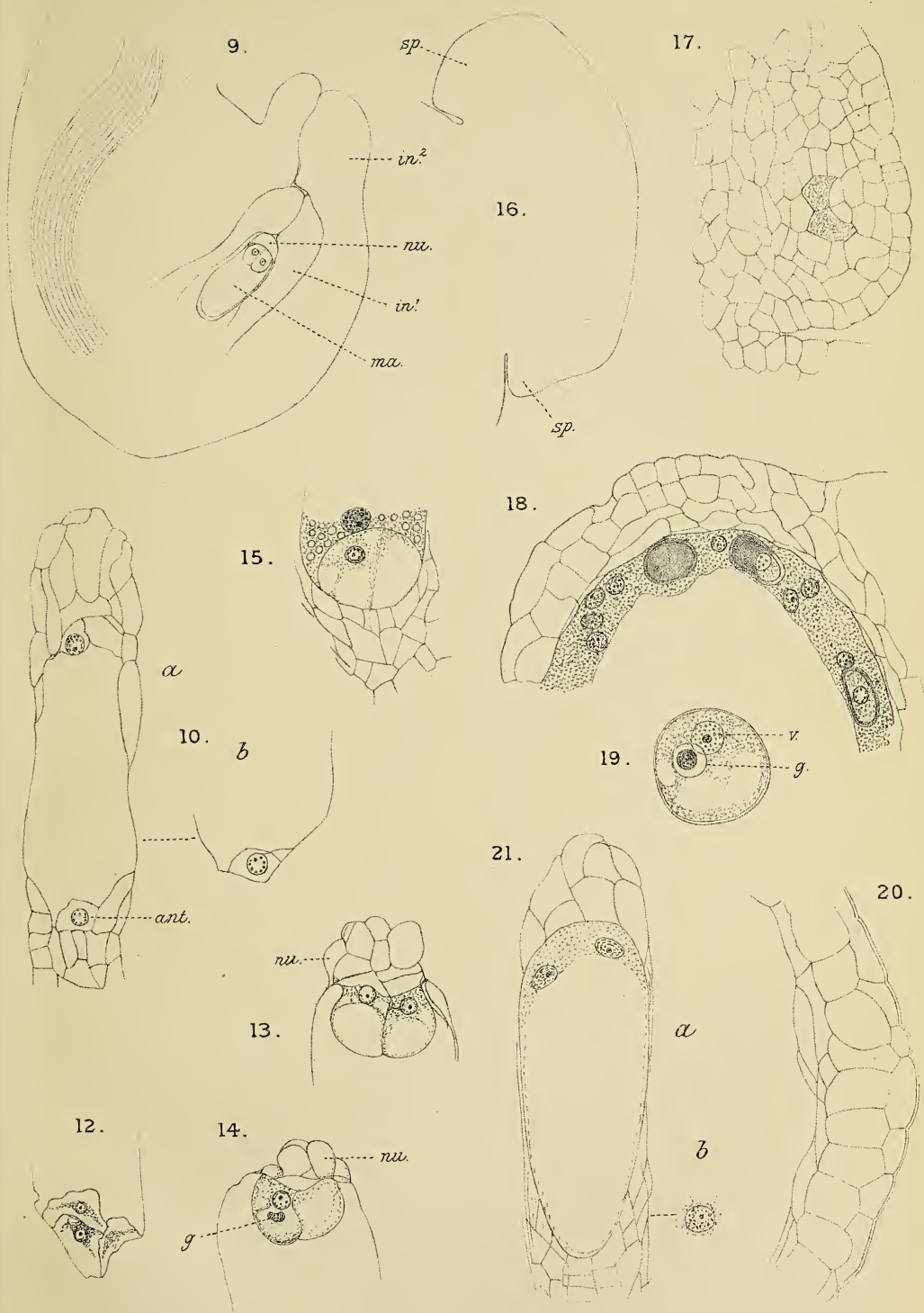
Fig. 56. Longitudinal section of a ripe ovule of *Philodendron lacerum*, Schott. Leitz, oc. 3, obj. 3.





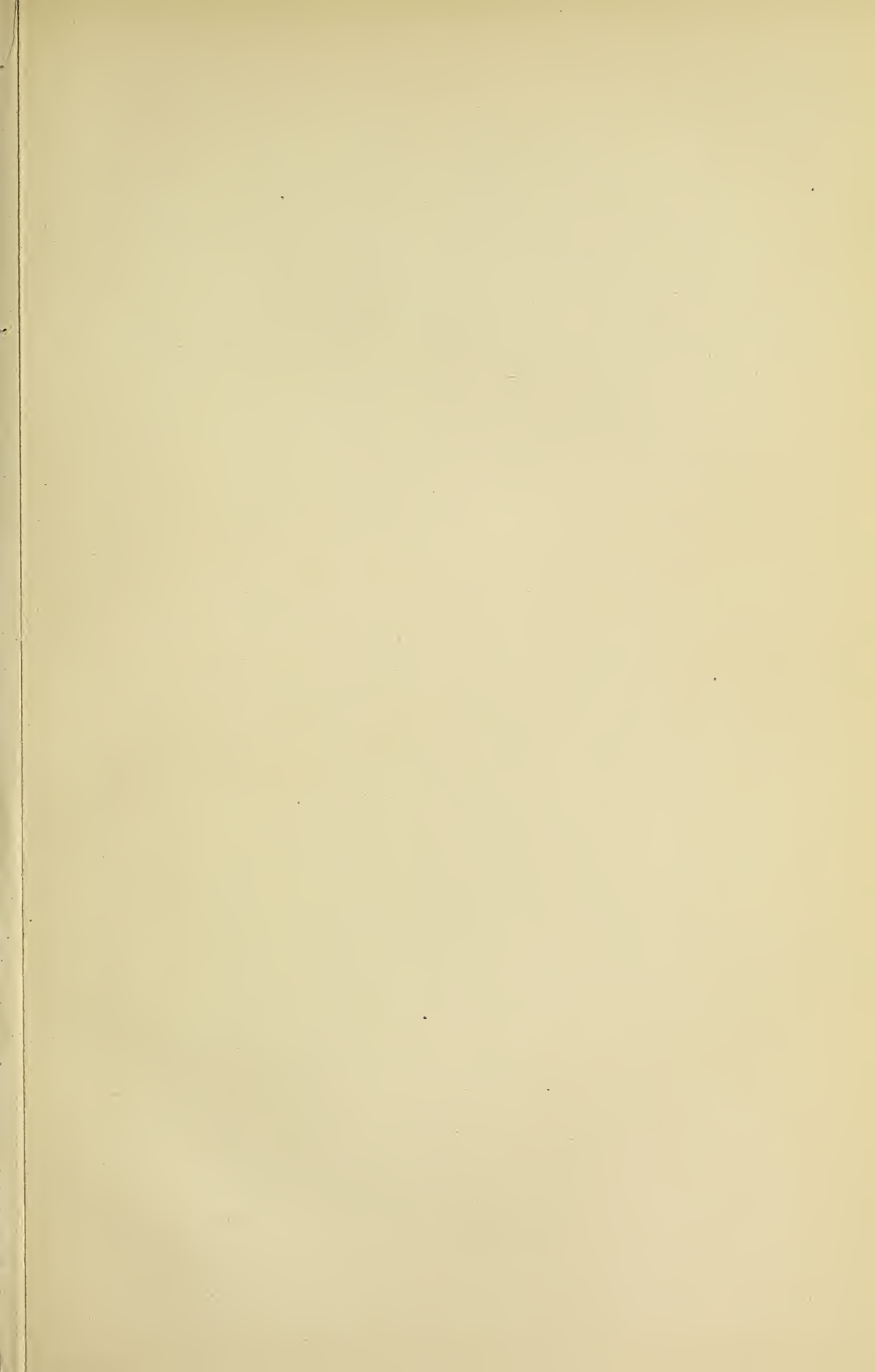


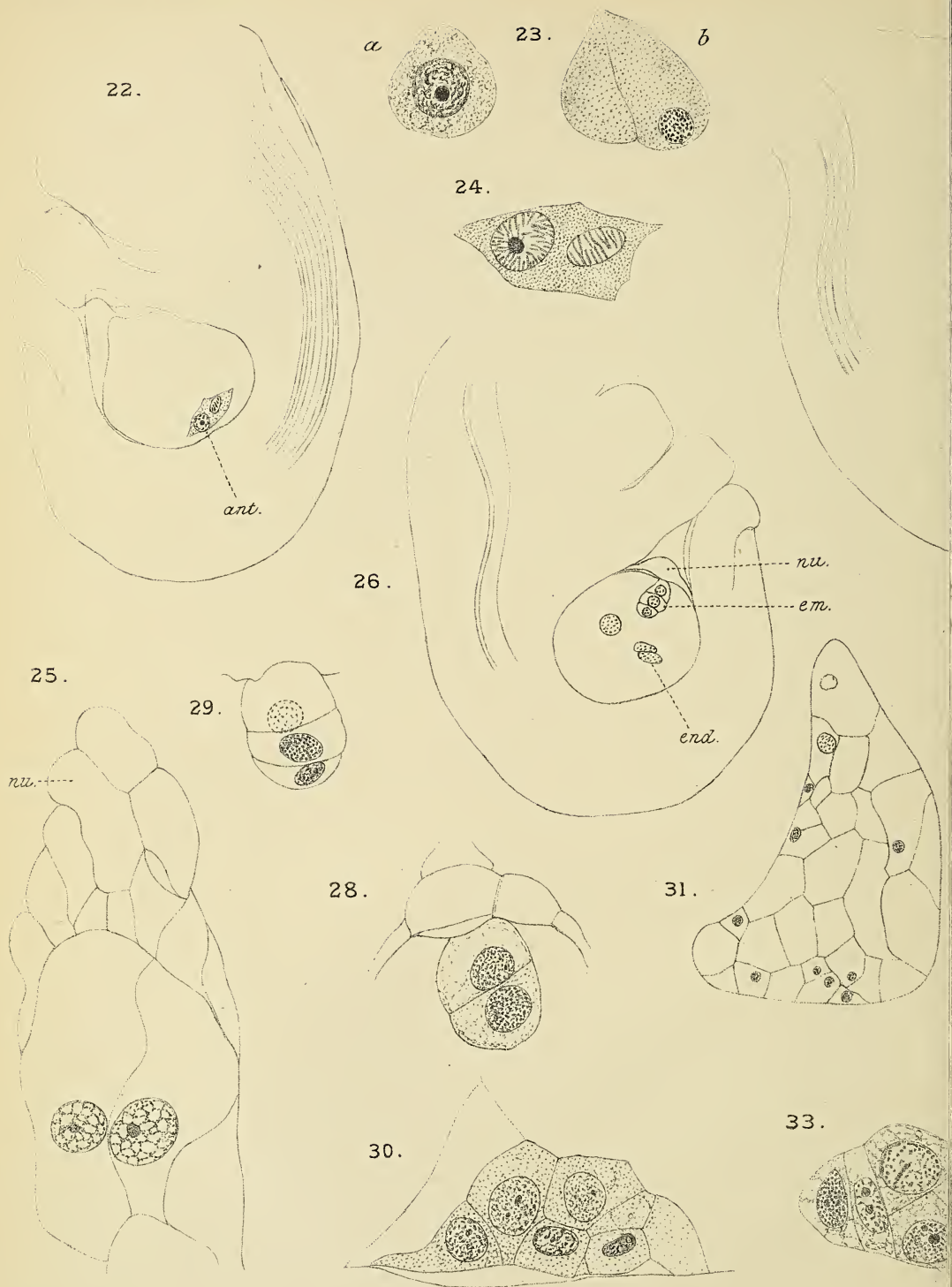
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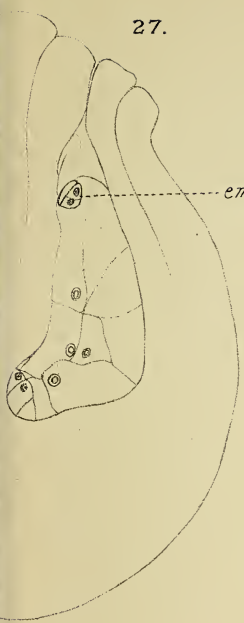




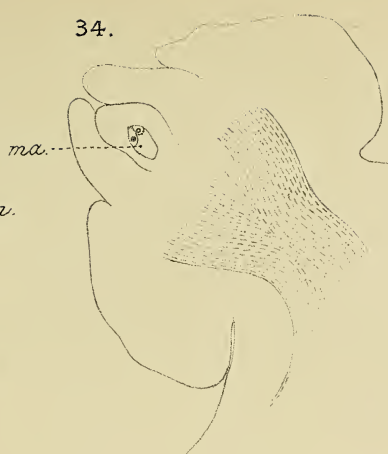


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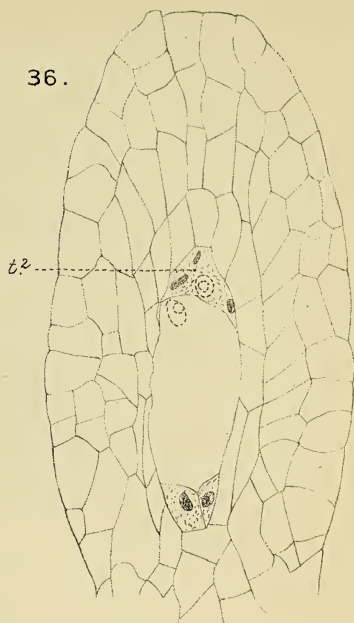
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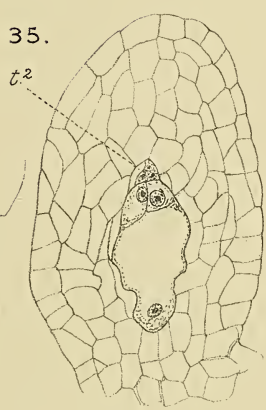
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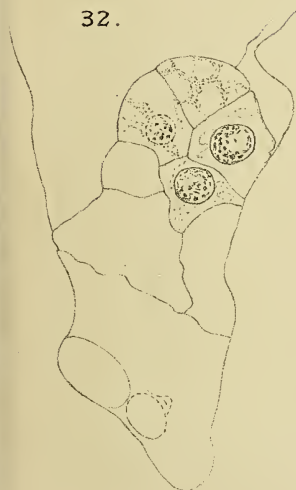
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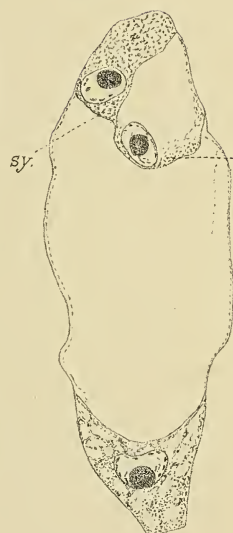
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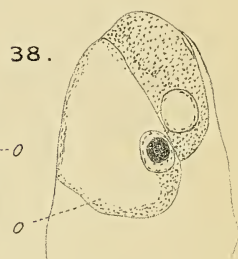
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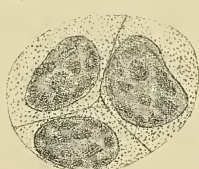
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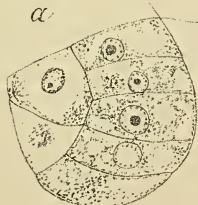
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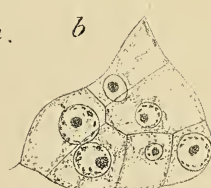


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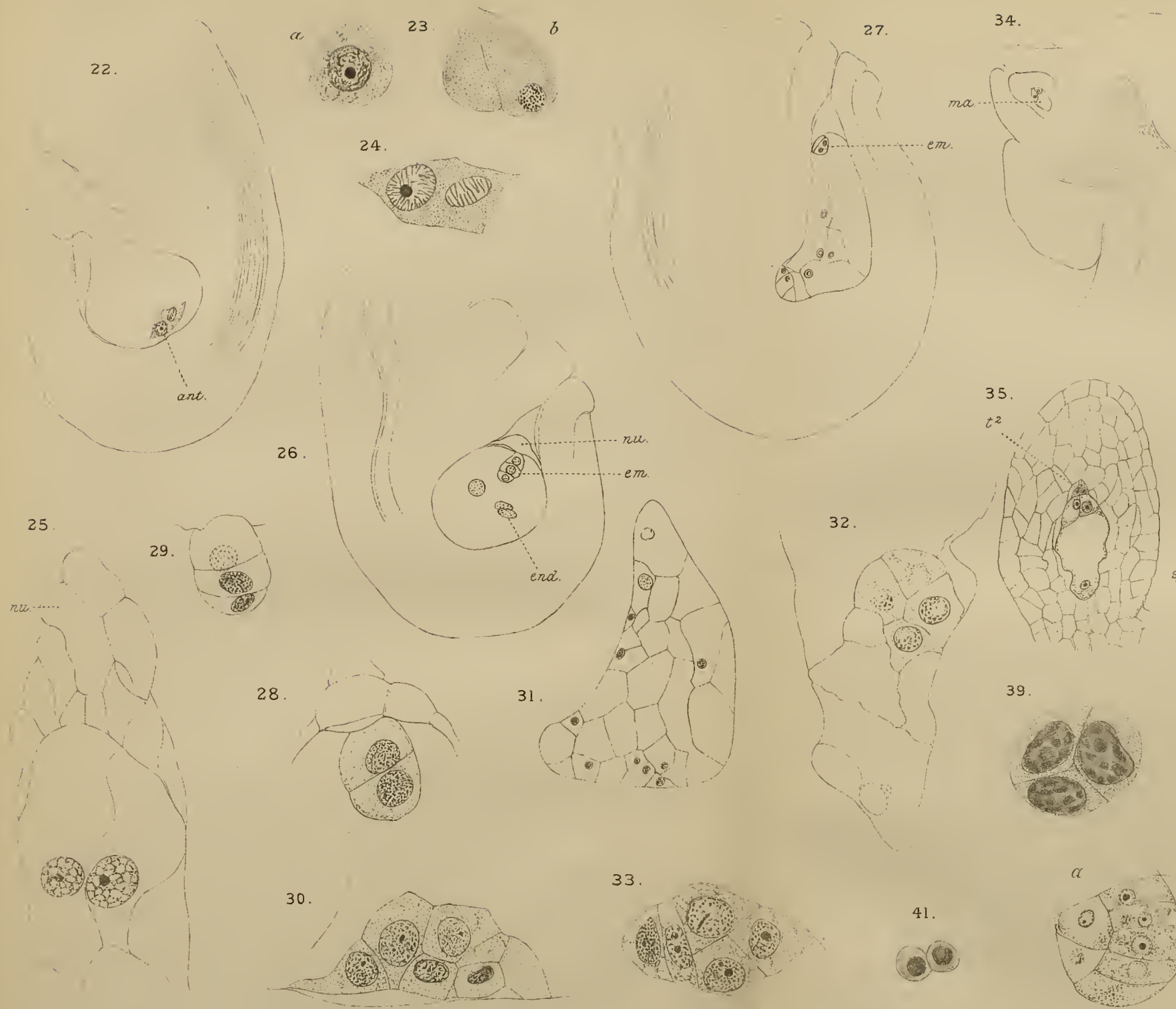
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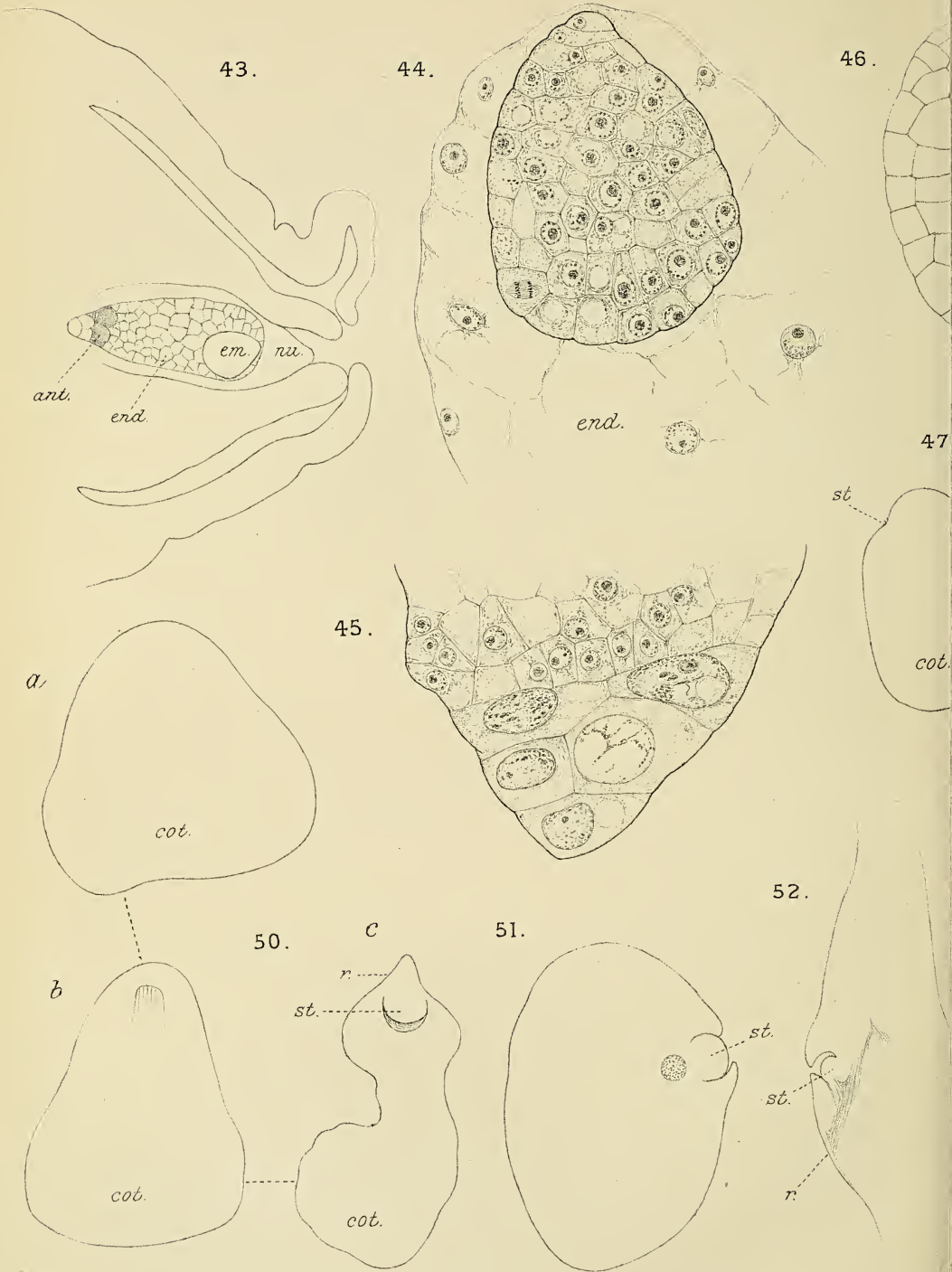


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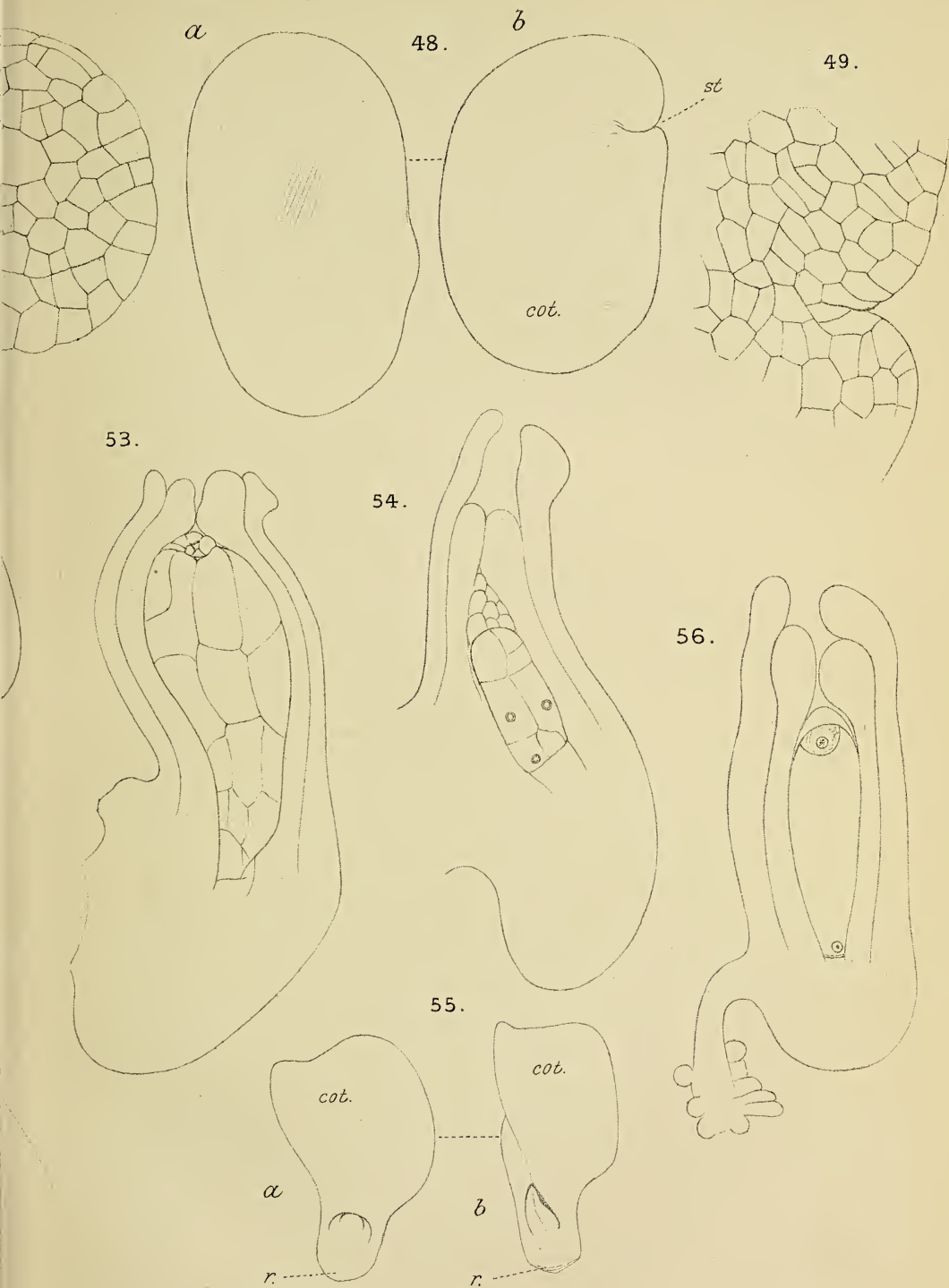






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CAMPBELL. — ON ARACEAE.





## On a Disease of Tradescantia.

BY

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With Plates IV and V.



DURING the summer of 1899 it was found that two species of *Tradescantia*, viz. *T. fluminensis* (Vell.) and *T. zebrina* (Hort.), growing in the greenhouses of the University Botanical Gardens, were suffering from an epidemic disease which showed all the symptoms of attack by a Fungus. In some of the houses the host-plants were completely killed off over considerable areas, while in others the plants, although obviously infected, nevertheless continued their growth in a more or less normal manner. It was noticed that many of the dead leaves and stems, in addition to showing dark spots and patches, were covered with long white conidiophores of a *Botryosporium* (Fig. 10), and also that dead leaves which did not show these structures speedily became covered with them when placed on damp blotting-paper under a bell jar. It appeared possible that this Fungus was the cause of the disease, and it thus became necessary to obtain pure cultures to follow out the complete

[Annals of Botany, Vol. XIV. No. LIII. March, 1900.]

development under the microscope by the hanging-drop method, and subsequently to attempt infection experiments on healthy host-plants.

In addition to the above symptoms, many of the still green leaves showed pale areas, as if the chlorophyll was being decolourized, and on some of these the microscope showed minute brown spots and, here and there, fungal hyphae.

Preliminary plate-cultures were made, using a food-material consisting of ten per cent. saccharose-gelatine, containing yeast-extract to supply the necessary mineral food materials. This medium was only used for one series of plates, as it was found to contain dead yeast-cells, and was consequently unsuitable for use in hanging-drops—it being difficult to determine when the drop contained a single spore of the Fungus under investigation. Subsequent cultures were made with ten per cent. saccharose-gelatine containing Klebs's solution:—

Potassium nitrate	2	grams	per	litre.
Magnesium sulphate	1	"	"	"
Potassium phosphate	2	"	"	"

which was found to be suited to the needs of the Fungus. One of the third series of plates was found to be pure, and from this hanging-drops were prepared. Some difficulty was experienced in obtaining the Fungus free from a species of *Cladosporium* or *Hormodendron*—a significant fact in view of subsequent observations—the peculiarities of which were studied later, and will be described below.

In the hanging-drops and on the plates the spores germinated in less than twenty-four hours, and in thirty hours colonies were visible with the naked eye on the first of a series of plates. In four days conidiophores were visible. The details of germination are shown in the drawings (Figs. 1–3), and those of spore-formation in Figs. 4–6.

The hyphae are septate and copiously branched, averaging 4–5  $\mu$  in diameter, and creeping horizontally: they throw up long conidiophores (Figs. 10, 11), each consisting of a

septate, cylindrical main axis, some  $10\mu$  or so thick and 1–3 or more mm. long, from which are put forth acropetally and at right angles, peg-like branches about  $5\mu$  thick in the middle, and  $50\mu$  or more long (Fig. 11). Each of these pedicels tapers below to its insertion in the axis, and widens above into a club-shaped head about  $10\mu$  thick. The head puts forth four or five irregularly swollen sterigmata, each about  $10$ – $12\mu$  long, whence clusters of spores (conidia) bud out, as in *Botrytis* (see Figs. 11 and 12). The conidia are ovoid or globoid, and average  $4$ – $5\mu$  in diameter. All the parts—mycelium, axis, heads, and capitula, and spores—are colourless, and glisten like silver or white floss silk in the mass, and the sum of the characters brings this Fungus into Corda's genus *Botryosporium*<sup>1</sup>.

The fertile hyphae grow down into the air below the drop and appear in the air darker than the submerged sterile hyphae. They vary in length between wide limits, are rarely branched, and give off lateral branchlets of equal length in a racemose manner as described. Each branchlet ends in a clavate swelling, which bears three or four circular appendages, which in turn bear the conidia, the whole forming a nearly globose head of spores.

During the formation of conidia the fertile hyphae were seen to bear globular bodies resembling gas-bubbles (Figs. 4, 5), the nature of which was not determined<sup>2</sup>. In many cases a head of conidia was seen to be encased in one of these 'bubbles,' and on several occasions, when such heads came in contact with the cover-slip, the 'bubble' burst, and the conidia were thrown some distance. It would thus appear as if these bodies are useful in spore-distribution. Such liberated spores were found to have a small circular body attached to one end, and it appeared probable that this structure served as an abjunctor to free the spore from the

<sup>1</sup> See Saccardo, *Sylloge Fungorum*, Vol. iv, p. 54, and Massee, *Brit. Fungus Flora*, Vol. iii, p. 291.

<sup>2</sup> Such gas-bubbles are very commonly found on conidiophores in the act of developing spores. The gas is probably enclosed in a slimy matrix; see Brefeld, *Unters. aus dem ges. Geb. der Mycol.*, Heft 10, p. 177 and Plate V, Fig. 42.



conidiophore. In many cases these abjunctors were seen between the conidia and conidiophores, and in such cases the conidia rapidly became free (Fig. 6).

In several instances a conidiophore fell into the film of water on the surface of the cover-slip, and at once ceased to form spores, the tip growing into an ordinary sterile hypha. Later, the tip grew down into the air, and again gave rise to branchlets and conidia, thus bearing out Klebs's observation<sup>1</sup> that conidia can only develop in relatively dry air, and are converted into ordinary hyphae if too wet.

After spore-formation had ceased, many of the sterile hyphae had brownish-yellow bodies, with rounded edges, attached to their surfaces (Fig. 14). These had the appearance of proteid crystals, but may have been mineral matters deposited from the concentrated and altered drop.

On the eleventh day it was found that the hyphae in the hanging-drops commenced to knot up in a remarkable manner, and on the twelfth day it was found that the hyphal contents commenced to aggregate in certain parts of the mycelium, leaving the rest empty (Fig. 7). This went on until finally thick-walled circular chlamydospore-like bodies containing granular protoplasm were found which afterwards showed budding (Fig. 8).

Cultures were next made with a food-material consisting of raisin-extract and gelatine. Vegetative growth was much more rapid and luxuriant than before, while the formation of conidiophores was delayed, especially on plate-cultures. After spore-formation had ceased chlamydospores were formed in a similar manner to that noted above (Figs. 8 and 9).

The Fungus was then grown by infecting sterilized blocks of the sap-wood of oak with spores from a pure culture. In the first instance an abundant feathery white mycelium was formed, and in seven days long white conidiophores made their appearance, and these speedily covered the wood.

The Fungus proved to be a species of *Botryosporium*, as

<sup>1</sup> Klebs, Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen, p. 452.



already stated, and the specific characters were found to agree most nearly with those of *B. diffusum*, Corda, occurring in rotten wood, branches, leaves, &c.<sup>1</sup>

It now became necessary to try infection experiments with healthy host-plants in order to test the parasitic nature or otherwise of the Fungus.

Preliminary experiments were made by placing a colony of the Fungus, with attached food-material, from a pure plate-culture, on both the upper and under surfaces of the leaves of apparently healthy host-plants growing in the greenhouses, and also out of doors. No infection took place, the leaves being kept under observation for fourteen days.

Next, a large quantity of infected *Tradescantia* material was collected, ground up with sand, and the extract filtered through a Pasteur filter. The filtered extract was then collected in minute capillary tubes, sterilized when drawn, the fine sealed ends of which were then broken as they were pushed into the tissues of the plants. Healthy plants infected with this extract did not show any ill effects, although as many as six capillary tubes were placed in each.

Afterwards infection experiments were made on a large scale, using healthy leaves to which a portion of the internode was attached, floating on water. Growing hyphae, germinating spores, or spores direct from the conidiophores, were sown on the upper and under surfaces of the leaves of both species of host-plants, the epidermis being left in the normal condition in some, or pricked with a sterilized needle in others. A similar set of experiments was made, in which a drop of food material was used in addition<sup>2</sup>, and in all cases blank experiments were performed to serve as a control. Only in rare instances did infection take place, and in all such cases the *entering hyphae were found to be olive-brown coloured and of a different character from the normal mycelium of the Botryosporium*. On the whole, the infection experiments demonstrated the non-parasitic nature of the Fungus

<sup>1</sup> See Massee, Brit. Fung. Fl., Vol. iii, p. 291.

<sup>2</sup> See Marshall Ward, A Lily Disease, Ann. Bot., Vol. ii, p. 355.

investigated, especially when it was borne in mind that plants from all the greenhouses, and also from out of doors, were used.

It was found in the case of the naturally-growing host-plants that infection started either on the upper surface of the leaf as a small semi-transparent dot or from the margin. Tangential sections of the upper epidermis containing one of these areas, and also of the infected margin, were made with a sterilized razor, and were placed in hanging-drops, with and without food-material. In all cases, the hyphae seen on the epidermis gave rise to the same species of *Cladosporium* as that mentioned above (p. 28) occurring as a weed in the early *Botryosporium* cultures (Figs. 15 and 16). From one of these drops pure plate-cultures were obtained, and the complete development was followed from a single spore in hanging-drop cultures under the microscope, as shown in Figs. 18–22.

After spore-formation the olive-brown septate mycelium appeared to be exhausted, no chlamydospore-formation being noted, as in the case of the *Botryosporium*, but any joint of the dark olive mycelium may serve as a chlamydospore-like body, and reproduce the Fungus vegetatively. The spores were liberated by a spherical abjunctor-like body similar to that noted in the case of the *Botryosporium*.

The *Cladosporium* was next grown on sterilized oak sap-wood, by infection with spores from a pure culture. Dark olive patches were produced, which gradually spread over the substratum.

The spores were found to be ellipsoid in shape, simple, olive-coloured, 4 to 6  $\mu$  long and 2 to 3  $\mu$  broad. The hyphae varied in breadth from 4 to 6  $\mu$ .

As seen growing on the epidermis, or after some days in culture, the mycelium is cut up into short segments, the older of which are thick-walled and dark olive-brown in colour. These segments may be barrel-shaped or globoid, and from 5 or 6 to 10 or 12  $\mu$  in diameter or more. They tail off into the thinner colourless hyphae with segments

3 to  $4\mu$  thick and 16 to  $18\mu$  long (Fig. 15). Any of the older segments, which have oily-looking drops in them, may serve as new centres of germination, acting like chlamydo-spores, as already stated.

The conidiophores are borne in immense numbers on the well-nourished mycelium, and present an extraordinary resemblance to *Penicillium* at first sight, a feature quite in accord with the earlier name of at least one member of the genus<sup>1</sup>. Each conidiospore consists of a pedicel of a few segments, which apparently branches in a pseudo-dichotomous manner, and bears the conidia at the tips of the ultimate branches. The totally different mode of origin from those of *Penicillium* is shown in Fig. 21.

Infection experiments were now performed on healthy host-plants and on leaves floating on water. In the first instance, a drop of food-material containing spores from a pure culture was placed on the upper surface of floating leaves. A control experiment, when no spores were used, was made at the same time. In three days all the leaves on which spores had been placed showed infection through the cuticle. No infection took place in the control leaves. Next, food-material and spores were placed on the upper and under sides of the leaves of growing host-plants, the upper epidermis being pricked with a sterile needle in one set of experiments. A control plant was used in each case. In a week all the leaves showed infection where spores had been sown, while the control plants, with the exception of one leaf, showed only negative results.

Stomatal infection was rare, the hyphae preferring to penetrate the middle lamella or even a guard cell. The points of infection were easily apparent by the dark-brown discolouration of the epidermis. These experiments were repeated on floating leaves, the result confirming the above.

Lastly, a series of experiments were made on floating leaves where spores were sown on the wounds formed by

<sup>1</sup> See Loew's description of *Penicillium Cladosporioides* (now *Hormodendron*, Sacc.) in Pringsh. Jahrb., Bd. vii, p. 494.



pricking the upper epidermis. No spores were sown on the control leaves. In three days infection took place, the spores sending out hyphae which passed down into the leaf. (See Fig. 23.)

As regards the systematic position of this Fungus, we are again—since no higher form of fructification could be obtained—driven to regard it as one of the Hyphomycetes or *Fungi Imperfecti*; and of the four great groups into which these are divided, the present form clearly comes into the second one, viz. the *Dematieae*, characterized by the dark-coloured hyphae and conidia, and the absence of any fasciculate stipes or ‘sporodochium’<sup>1</sup>.

Now appears a difficulty, however. If we follow the classification logically, the present Fungus falls naturally into section I, *Amerosporeae*, because the elliptical or oblong conidia are continuous, i. e. non-septate; but it is evident on comparing it with *Cladosporium* (which Saccardo puts into section II, the *Didymosporeae* with typically uni-septate spores) that our Fungus comes into that or a closely allied genus.

Adhering to the classificatory scheme of the Sylloge, however, we place it in the second subsection (*Macronemeae*) of the *Amerosporeae*, and so get to the genus *Hormodendron*.

A good deal of light is thrown on the matter by a paper published by Schostakowitsch in 1895 on the conditions of conidia-formation in the Sooty Moulds<sup>2</sup>, where he shows pretty clearly that *Hormodendron cladosporioides*, Sacc., is practically *Cladosporium*, although the author himself attempts to uphold the individuality of both. This seems hardly possible in view of his own admission, on p. 369, that on agar ‘*Cladosporium* bildet hier eine Form, die man sehr schwer von *Hormodendron* unterscheiden kann,’ and the comparison of his own figures on pp. 366 and 368 points also to the practical impossibility of maintaining the autonomy of the two genera by characters of any utility.

<sup>1</sup> See Saccardo, Sylloge Fungorum, Vol. iv.

<sup>2</sup> Ueber die Bedingungen der Conidienbildung bei Russthaupilzen, Flora, Bd. lxxxi, 1895, p. 362.



Nevertheless, other writers have insisted also on the autonomy of the genus *Hormodendron*, of whom Bruhne<sup>1</sup> is especially worth noting, since the form he describes is parasitic on Barley with symptoms almost identical with those of the present disease.

Laurent<sup>2</sup> and Costantin<sup>3</sup> both maintain that *Hormodendron* and *Cladosporium* are merely forms of one and the same genus; Janczewsky<sup>4</sup>, on the other hand, failed to obtain *Hormodendron* from *Cladosporium* or vice versa.

The whole controversy—in so far as it concerns these two genera—really seems to turn on one or two trivial points of difference, as already indicated, and of which the following are further illustrations.

Schostakowitsch (l. c. p. 367) says the spores of *Hormodendron* have smooth surfaces, those of *Cladosporium* being warted: nevertheless Bruhne figures the conidia of his *Hormodendron* (l. c., Plate I, Fig. 2) with warts, and expressly states (l. c. pp. 5 and 6) that they are *smooth or warted* according to the different conditions of culture.

Again, Schostakowitsch (l. c., p. 370) says that *Cladosporium* ‘besitzt die merkwürdige Eigenschaft, bei 0–2° C. seinen vollen Entwicklungsgang zu durchlaufen . . . Die Hormodendronsporen vermögen unter solchen Bedingungen nur kurze Keimschläuche zu bilden.’ Nevertheless Bruhne (l. c., p. 31) shows that cultures of *Hormodendron* produced spores abundantly at temperatures ranging from 5.5° at mid-day to ‘einige Grade unter 0° C.’ at night, and the vessels were frozen.

It is obvious, therefore, that much remains to be done before we can regard this question as settled. Meanwhile, it is interesting to note that the course of events in the life-history of this Fungus on *Tradescantia*, and in the parasitism it displays, are extremely similar to those above referred to.

<sup>1</sup> *Hormodendron Hordei*, Zopf's Beitr. zur Phys. u. Morph. niederer Organismen, Heft 4, 1894, p. 1.

<sup>2</sup> Recherches sur le polymorphisme du *Cladosporium herbarum*, Ann. de l'Inst. Pasteur, Vol. ii, 1888.

<sup>3</sup> Sur les variations des *Alternaria* et des *Cladosporium*, Rev. gén. de Bot., 1889.

<sup>4</sup> Zur Entwicklungsgeschichte von *Penicillium*, Pringsh. Jahrb., Bd. vii.

*Cladosporium* is by no means unknown as a parasite, although its easy culture as a pronounced saprophyte suggests that special conditions either of the host or the Fungus are necessary before it can attack living plants with such energy as to cause an epidemic.

Lopriore<sup>1</sup> has described an epidemic on Wheat caused by *C. herbarum*, and collected the literature to date.

Frank<sup>2</sup> quotes yet other cases where various Monocotyledons, fruit trees, olives, tomatoes, cucumbers, &c., are injured by other species of *Cladosporium*, and also brings the literature up to date, showing at the same time what enormous confusion exists as to the connexion between this and other genera.

The difficulties of confusion are increased when we find that Brefeld<sup>3</sup> describes as the conidial form of *Microsphaerella* a Fungus of essentially the same type as that under discussion, and it is evident that nothing final can be done before we trace the more perfect stages.

Brefeld refers to this type as the *Ramularia* form, and quotes Tulasne's Fig. 7, Table XXXI, in the *Carpologia*, which certainly bears out his comparison. The doubt arises, however, whether Tulasne did not draw more than one Fungus in his figure. Tulasne<sup>4</sup> also refers this type to *Pleospora*.

Hence it is evident that a long research will be necessary to clear up the numerous difficulties accumulated around the Fungus *Cladosporium* and its allies.

In conclusion, I wish to thank Professor Marshall Ward, under whose constant supervision this work has been carried out, for much kind assistance and advice.

CAMBRIDGE BOTANICAL LABORATORY,

September 4, 1899.

<sup>1</sup> Die Schwärze des Getreides, Ber. d. deutsch. bot. Ges., 1892, Bd. x, p. 72.

<sup>2</sup> Die Krankheiten der Pflanzen, 2nd ed., 1896, p. 315, &c.

<sup>3</sup> Unters. aus dem ges. Gebiete, &c., Heft 8, p. 213, and Plate VI, Figs. 38-42.

<sup>4</sup> *Carpologia*, Vol. ii, Plate XXXII, Figs. 13 and 14.

## EXPLANATION OF FIGURES IN PLATES IV AND V.

Illustrating Mr. Howard's paper on a Disease of *Tradescantia*.

Fig. 1. Three spores of *Botryosporium* germinating in a hanging-drop of nutrient gelatine. The sowing was made July 29 at 1 p.m., temp. =  $21-22^{\circ}$  C. throughout.  $a$  = at 12.15 a.m. July 30;  $b$  = 12.35 p.m. July 30;  $c$  = 12.25 p.m. July 30.

Fig. 2. The same a few hours later.  $a'$  = 2.30 p.m.;  $b'$  = 3.15 p.m.;  $c'$  = 3.25 p.m. July 30.

Fig. 3. The same later still.  $a''$  = 6.40 p.m.;  $b''$  = 7.45 p.m.;  $c''$  = 7 p.m. July 30.

Fig. 4. Three stages in the development of the conidiophore and conidia from a mycelium six days old.  $a$  = Aug. 4, 10 a.m.;  $b$  = 11.45 a.m.;  $c$  = 2.30 p.m. same day. The  $\times$  is in a gas bubble which remained throughout.  $t = 22^{\circ}$  C. Zeiss BB.

Fig. 5. Similar stages of an older conidiophore under  $\frac{1}{6}$ , showing the development of the conidia.  $a$  = 11 a.m.;  $b$  = 12.40 p.m.;  $c$  = 6.20 p.m.

Fig. 6. Two heads of the conidiophore, showing the abjunction of the spores, each of which has a minute drop-like body at its proximal end—the 'disjunctor'?

Fig. 7. Stages in the formation of chlamydospore-like segments.  $a$ , A piece of the mycelium of a hanging-drop culture several days old, on Aug. 10 at 10.15 a.m., showing the accumulation of the protoplasm in certain segments, leaving others empty. The segments marked  $x$  are the same throughout the series.  $b$ , The same on Aug. 11 at 10.45 a.m.;  $c$ , at 10.40 a.m. next day;  $d$ , at 11.15 a.m. Aug. 13;  $e$ , at 8.50 p.m. same day;  $f$ , at 11.30 a.m. Aug. 14. The parts with firmer walls and filled with protoplasm now pass over to a resting condition, the intermediate empty portions shrivelling up.

Fig. 8. Chlamydospore formation in a coiled piece of mycelium several days old.  $a$ , on Aug. 10 at 10 a.m.;  $b$ , twenty-four hours later;  $c$ , forty-eight hours later still. The lower part of the coil (at  $\times$ ) is drawn separately.  $d$ , twenty-four hours later than  $c$ . In addition to forming the chlamydospore-like gemmae, some of the segments in  $c$  and  $d$  are putting forth bud-like processes.

Fig. 9. Gemmation or budding of old mycelium.  $a$ , A piece drawn at 10 a.m. Aug. 21;  $b$ , the part marked  $\times$  a week later;  $c$ , the same Aug. 31, showing slow budding.

Fig. 10. Piece of the Fungus (*Botryosporium*) under low power to show habit.

Fig. 11. Portion of a conidiophore, showing septa, spicules, &c., under  $\frac{1}{6}$ .

Fig. 12. Terminal portion of spicules and spores under higher power.

Fig. 13. Portion of conidiophore under still higher power, showing septum with pit.

Fig. 14. Portion of mycelium, showing crystalline accretions, or possibly excretions of apparently proteid nature.  $\frac{1}{6}$ .



Fig. 15. Portion of a mycelium as found growing on the epidermis under  $\frac{1}{4}$ .

Fig. 16. Low power view of *Cladosporium* (*Hormodendron* form) with conidiophores to show habit.

Fig. 17. One of the conidiophores of last, more highly magnified and showing apparent dichotomous branching.

Fig. 18. Germination of spore of *Cladosporium* in a hanging-drop (made Aug. 25 at 9 p.m.) at 10.30 p.m. Aug. 26.  $t = 22.5^{\circ}\text{C}$ .  $\frac{1}{4}$ .

Fig. 19. The same next day, Aug. 27, at 11.30 a.m.  $t = 22.5^{\circ}\text{C}$ .  $\frac{1}{4}$ .

Fig. 20. The same at 3.30 p.m. under low power. At 6.15 p.m. aerial hyphae were emerging from the drop to form conidiophores.

Fig. 21. Stages in the development of one of the above conidiophores.  $a$  = at 6.15 p.m. Aug. 28,  $t = 23^{\circ}\text{C}$ .;  $b$  = at 10.30 p.m.,  $t = 22^{\circ}\text{C}$ ., several minute gas-bubbles have appeared on the elongated hyphae;  $c$  = at 12 p.m.,  $t = 22^{\circ}\text{C}$ ., the tip has grown forwards and been cut off by a septum;  $d$  = at 12.20 a.m.,  $t = 21^{\circ}\text{C}$ ., the terminal segment is budding out another segment at its apex;  $e$  = 12.35 a.m.,  $t = 21^{\circ}\text{C}$ ., another bud appears below the first septum ( $\times$ ).

This point ( $\times$ ) is marked as a fixed point throughout.  $f$ , the last-named bud is now (12.55 a.m.,  $t = 22^{\circ}\text{C}$ .) budding at its apex, and minute gas-bubbles appear at the surface of the older buds;  $g$  = at 1.10 a.m.,  $t = 23.5^{\circ}\text{C}$ . [N.B. The rise in temperature was due to the heat radiated from the lamp.]  $h$  = at 9.30 a.m.,  $t = 21.5^{\circ}\text{C}$ .

The conidiophore is now complete, and any segment acts as a spore. At the \* is another branch, the fate of which was further followed.  $i$  = this branch at 9.45 a.m.,  $t = 21.5$ . The large gas-bubble at the right hand grows larger.  $k$  = at 3.15 p.m.,  $t = 21.5$ . All under  $\frac{1}{4}$ .

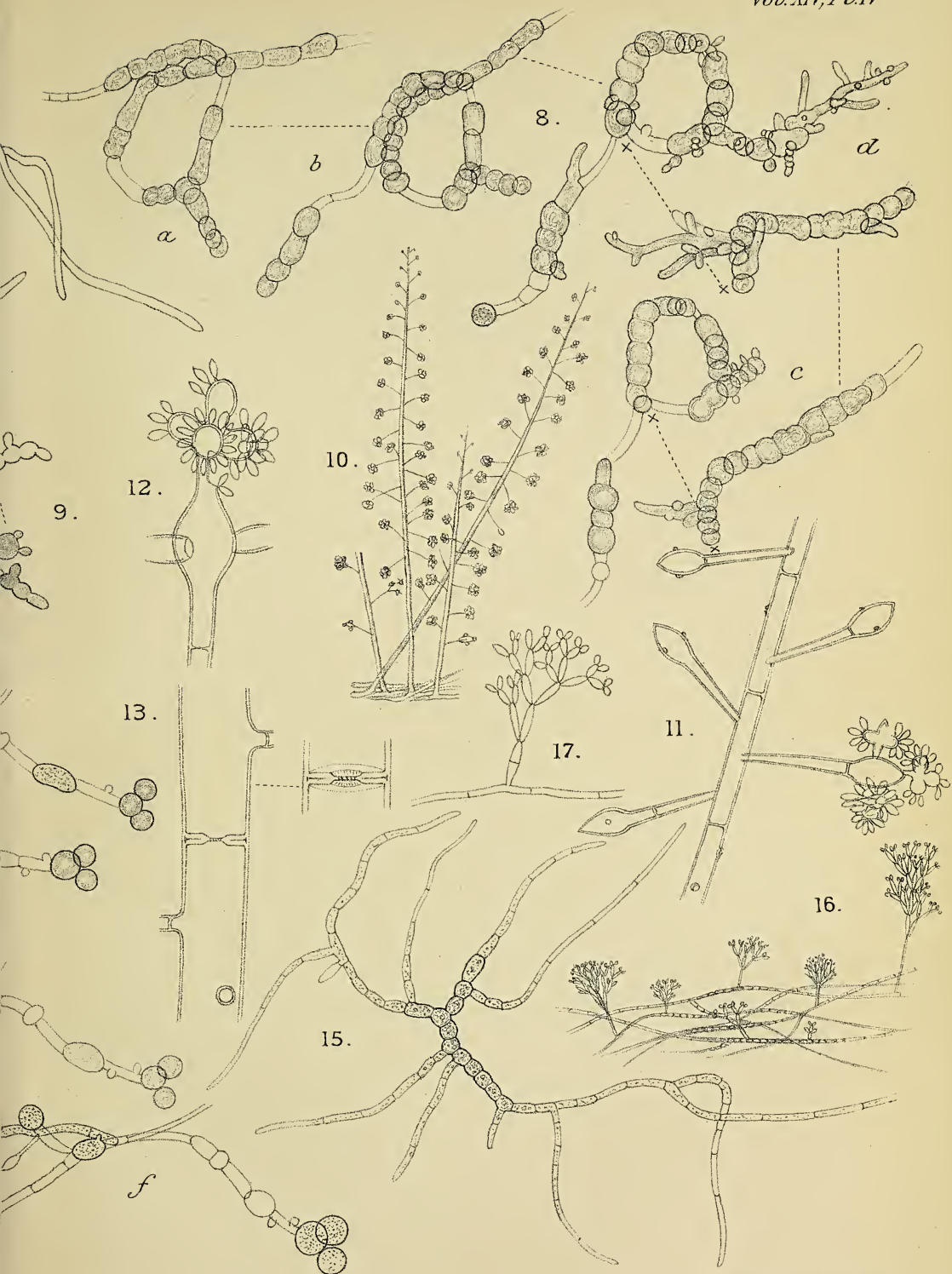
Fig. 22. Stages in growth of a branch from a mycelium twenty-four hours old in hanging-drop.  $a$ , at 9.30 a.m., Aug. 27,  $t = 21^{\circ}\text{C}$ .;  $b$ , at noon same day,  $t = 22.5$ . The vacuolation, beginning in  $a$ , is now more pronounced, and several septa have formed.  $c$  = at 2.45 p.m.,  $t = 22.5^{\circ}\text{C}$ .;  $d$ , the distal moiety of same at 4 p.m.,  $t = 23$ ;  $e$ , at 5.45 p.m.,  $t = 22.5$ . Next day the walls begin to thicken and turn brown, and in a day or two the short swollen segments (as in Fig. 13) predominate.  $\frac{1}{4}$ .

Fig. 23. Portion of epidermis, seen from within, showing penetration of infecting hypha. The part in paler outline is the germinating spore on the outside: the portions inside are running in the middle lamella.



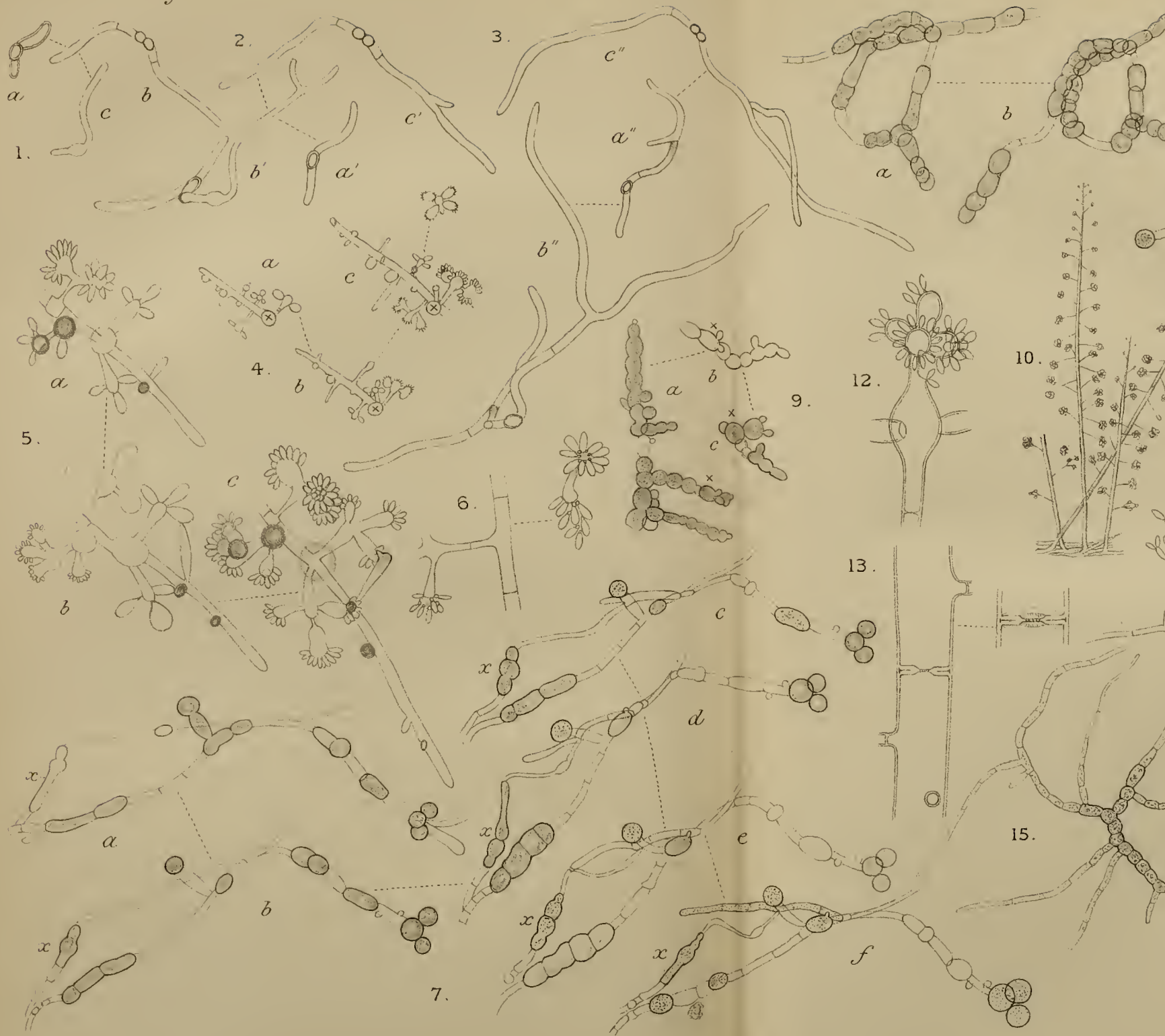






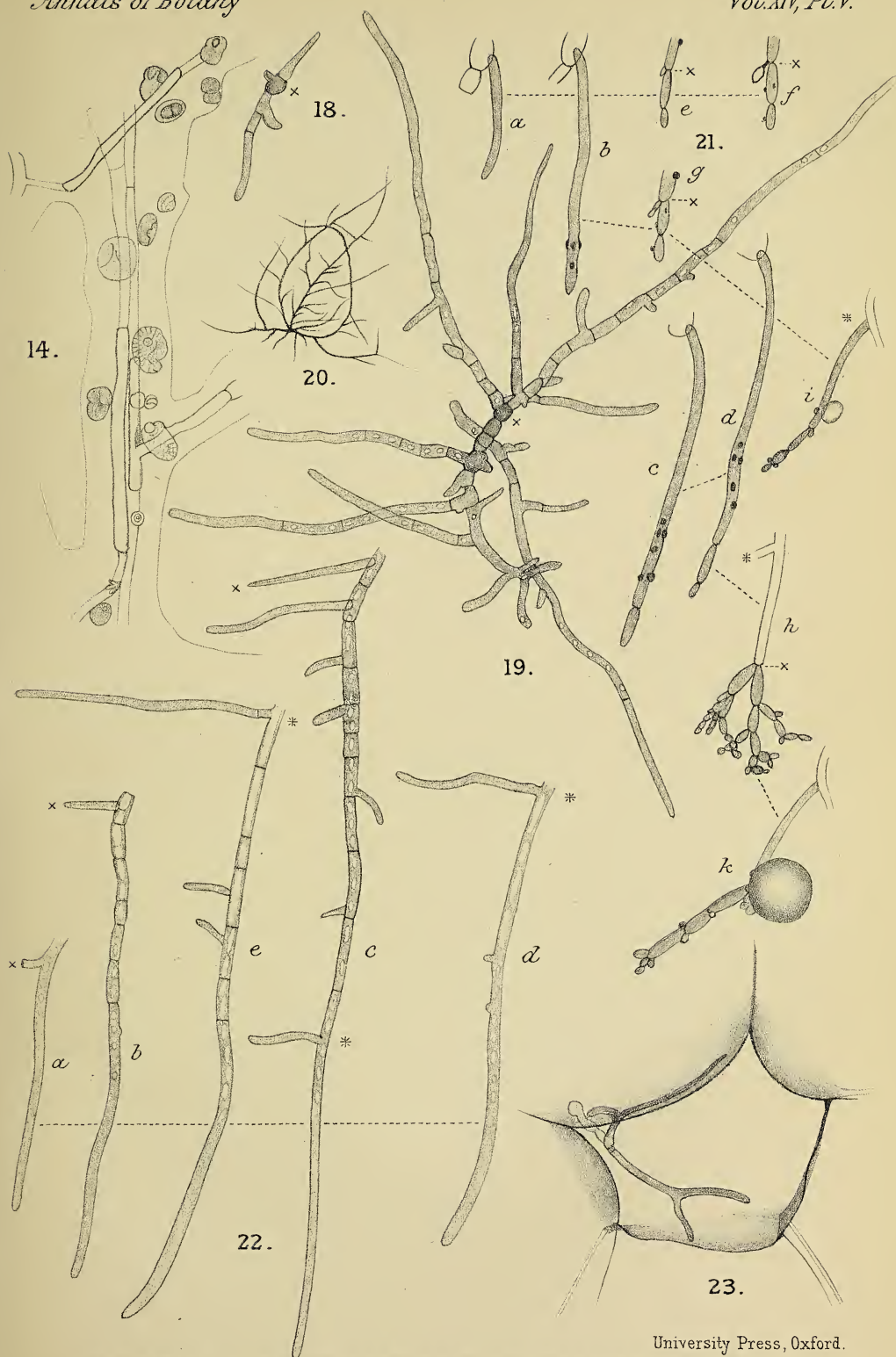






HOWARD. — ON A DISEASE OF TRADESCANTIA.









# The Structure of the Female 'Flower' in Coniferae.

An Historical Study.

BY

W. C. WORSDELL, F.L.S.



With seven Figures in the Text.



## INTRODUCTION.

THERE are few structures in plants which, to the ordinary observer, appear at first sight more simple or more easily understood than the female reproductive parts of the great order Coniferae. Yet the problem of the true morphology and structure of these organs has taxed the mental and observational resources of expert investigators for a longer period of time than, and to as great a degree as, any other botanical question of a similar nature. And still, at the present time, the nature of these organs, for the majority of botanists at any rate, remains the unsolved problem that it was at the very beginning of the century.

It is highly instructive to the modern morphological student to cast a retrospective look at the various views on the question which, for more than a century and a half, have from time to time been put forward by the botanists of France, Germany, Denmark, Sweden, Austria, Italy, America, and this country.

[Annals of Botany, Vol. XIV, No. LIII. March, 1900.]

In the following pages I shall only consider the most important and striking of the many views advanced by those authors who have expressed them in the most elaborate and authoritative manner. At the end, however, will be found a bibliography of the subject as complete as I have been able to compile, which, in any case, will be amply sufficient to afford information regarding the history of the whole subject.

The *male* reproductive organs of Coniferae are comparatively simple in structure and morphology, strangely unlike, in these respects, those of the opposite sex. But a comparison between the two cannot be instituted until some attempt has been made to unravel the structure and morphology of the female 'flowers.'

These organs are, in different genera, greatly dissimilar as regards their superficial aspect, a fact which renders all the more obscure their real nature and relationships. We have only to think of the female parts of the Pine, Cypress, Juniper, and Yew, in order to become impressed with this fact. As the various genera of the order Coniferae are manifestly more intimately allied the one to the other than any one of them is either to the Gnetaceae or the Cycads, it is obvious that it would be useless to investigate the structure and morphological nature of any one of these genera independently of all the rest; they must, on the contrary, be all considered, in this respect, interdependently, by direct comparative study of their respective parts, if we are to arrive at any worthy conclusion as to their real significance. The nature of the cone of an *Abies* cannot be considered along these lines apart from the 'berry' of a Yew or a Juniper, for parts which are obscure in their meaning in the one form are often rendered intelligible by their more easily explicable counterpart in the other, with regard to the relative positions of parts or organs, their mode of development, &c. For a full attempt at a solution of the problem it would also be requisite to institute a comparison of the Coniferae, both individually and as a whole, with the allied

orders of Gnetaceae and Cycadaceae, as also with the most nearly related fossil plants.

In the Abietineae the female organs are arranged in elongated cones, very compact and woody in character when mature. Their bulk is largely made up by large, woody scales, usually

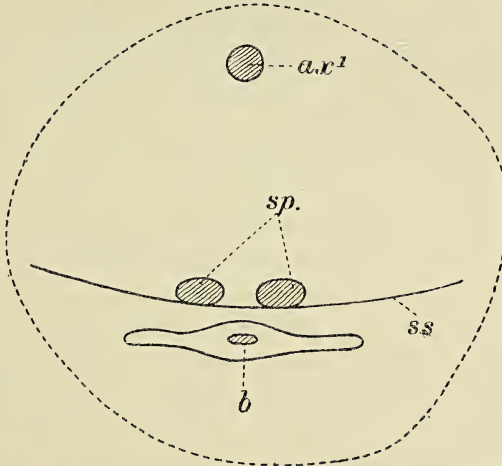


Fig. 1. Diagram of normal 'flower' of Abietineae.  
*b* = bract; *ss* = seminiferous scale; *sp* = sporangia;  
*ax¹* = primary axis of cone.

thickened and enlarged externally and known as the 'seminiferous scales,' each bearing on its upper surface at the base two inverted sporangia. The seminiferous scale is in the axil of a very much smaller bract, with which it is usually fused at the very base (Fig. 1).

In the Araucarieae there is but a single scale representing the bract, with (in *Araucaria*) an outgrowth resembling a ligule on its upper surface, and a single sporangium enclosed within

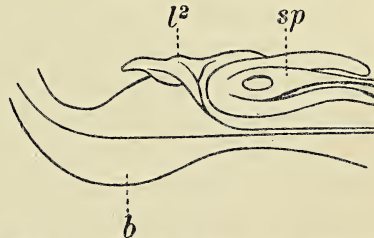


Fig. 2. Diagram of 'flower' of *Araucaria*.  
*b* = bract; *l²* = ligule-like seminiferous  
scale; *sp* = sporangium. (After Eichler.)

the tissues of the scale, and situated some distance above

its base (Fig. 2). In *Agathis* there is no sign of any ligular outgrowth.

In the Cupressineae the scales are not arranged spirally in elongated cones but in whorls. Here also there is but a single woody scale, which has, however, two distinct apices or tips at its swollen anterior end. In *Juniperus* the scales composing each whorl become fleshy and fuse laterally to form a berry, so that the seeds become enclosed. Each scale in this genus has but one seed; in *Cupressus*, however, there may be very many to each scale.

In the Taxodieae the structure of the scale is the same as that in Cupressineae.

In Taxeae a single sporangium is borne at the end of a secondary axis furnished with three pairs of bracts. The sporangium is attached to no scale, but the seed or mature sporangium is enclosed by a fleshy, coloured aril.

In *Podocarpus* the anatropous ovule is fused with the bract and extends far beyond the latter, owing to its being carried up on a long stalk. It has two integuments, of which the outer is fleshy and coloured. In some species the bracts and axis are fused into a coloured, succulent whole.

The above are the principal types of structure to be met with in this order. I have simply described the structures according to their bare, superficial appearance; and will now proceed to reproduce the various views and ideas as to the significance and true interpretation of these structures.

At the outset I may state that the whole question and controversy turns on two main points: (1) the nature of the 'seminiferous scale'; and (2) that of the sporangial envelope; these two factors are intimately bound up with one another, and neither can be in any way ignored when treating of the other, if an attempt at any thorough elucidation of the problem is to be made.

#### HISTORICAL SKETCH.

As is naturally to be expected, the older botanists approached the question in what, to our modern notions, is



a very rough and ready manner. Their ideas about comparative morphology may often seem crude when placed in comparison with those of to-day, and as they judged primarily by outward appearances when classifying and comparing organs, it is small wonder if we find that those ideas misled them with regard to many points.

Long before the middle of the last century Linnaeus (3) had already attempted the solution of this botanical puzzle, which to-day is as much an enigmatic chaos for the majority of students as it was in his, the dawning age of botany. Linnaeus considered that the seminiferous scale of Abietineae represented a calyx; that there was no corolla; and that each ovule represented a pistil consisting of a very small ovary with simple style and stigma which became changed when ripe into a winged nut. In *Juniperus* he describes the three exterior scales of the berry as a calyx and the three interior as a corolla.

Jussieu (6), writing towards the close of the same century on the female organ of the Abietineae, mentions an outer scale bearing two pistils on its base and two glandular stigmas; and an inner scale, formed on ripening of the fruit, bearing two one-seeded, winged capsules. His deduction was that the bracts were styles from their caducous nature, and that the seminiferous scale was a bilocular ovary.

Mirbel (17, 18, 20), at the very beginning of the present century, thus describes the female organs of this order: 'The Pines, Spruces, Larches have female catkins whose flowers, hidden in cupules, are inverted so that the stigmas are directed towards the axis, and whose broad peduncles, inserted each at the base of a bract, become insensibly transformed into woody scales, covering the ripe fruits, and forming a cone or strobilus by their approximation.' Here, what the majority of botanists now call the 'integument' is for him a 'cupule,' our 'nucellus' is his 'flower,' and our 'seminiferous scale' is his 'peduncle.' Further: 'The Cypresses, the *Thujas*, the Junipers, and *Schubertia* [*Taxodium*], have also a kind of catkin. Its axis is very short, its flowers erect, and enclosed

in cupules inserted immediately on bracts which become woody (Cypress, *Thuja*, *Schubertia*) or succulent (Juniper), and whose apices expand into a structure like a nail-head. These scales compose what some modern people call galbules.' But at a much later date (1843) he somewhat modifies this view, for he writes (of *Pinus*): 'These are assuredly the simplest flowers known; they consist of a conical nucellus contained in an open ovary.' And of *Thuja*: 'It consists simply of an ovary and a nucellus; but this flower is erect, whilst in the Abietineae it is inverted.'

L. C. Richard (24), in 1826, held very much the same view as Mirbel, for he says: 'In all Conifers the female flowers consist essentially of a pistil and a simple perianth or calyx.' In all Conifers, except *Taxus* and *Agathis*, he always recognizes the presence of a double scale. As regards the fruit, he writes: 'The ovary is semi-inferior, owing to its lower part being united with the calyx, which latter then necessarily takes part in the formation of the fruit to form the epicarp. The wall of the pericarp is always very thin, and so adherent to the integument of the seed that they seem to form a single membrane applied to the "amande." The fruit of Coniferae is a true caryopsis or a sort of nut, i. e. a unilocular, one-seeded, indehiscent fruit, whose pericarp is intimately fused with the integument of the seed.' In *Podocarpus* the calyx consists of an inner, bony, and an outer, fleshy portion. The aril of *Taxus* and *Phyllocladus* he describes as a special organ, an extraordinary development of the receptacle. Finally, he divides the order into two groups—the Podocarpeae, Taxeae, and *Ephedra*, possessing simple fruits; and the Cupressineae, Abietineae, and Araucarieae, possessing compound fruits. He compares the flowers of Coniferae with those of the Amentaceae.

The eminent English botanist, Robert Brown (25), emerges conspicuous from among the investigators of the first half of this century, as the first exponent<sup>1</sup> of the theory which now, seventy years after, is most favoured by botanists, viz. that

<sup>1</sup> But Ray, in 1682, held that the ovules were naked.

of *Gymnospermy*. In support of this idea, he compares the female organs of Coniferae with those of Cycads (which latter are for him evidently ovules), and with the ovules of other Phanerogamous plants. The proof lies chiefly in the resemblance between the body lying within the envelope and the nucellus of a typically-constructed ovule. The seminiferous scale is an *open carpel* bearing ovules at its base.

Schleiden (34, 36) is noted as the exponent of the view, first of all put forward in 1839, and later in his 'Grundzüge der wissenschaftlichen Botanik' in 1843, that a *placenta* is always an *axial* structure. He cites the Cupressineae and Taxineae as examples of orders with basilar ovules in which the placenta is the extremity of the axis, and the Abietineae as amongst those in which the placenta is an organ distinct from the carpellary leaves, arising later than the latter. 'In Abietineae the scale, considered by R. Brown as an open ovary, is only the axillary bud of the carpellary leaf, situated

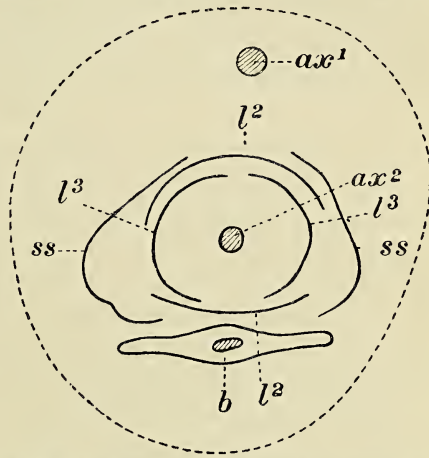


Fig. 3. Diagram of an abnormal 'flower' of Abietineae; the seminiferous scale (*ss*) is split into the two first leaves of an axillary shoot; *b* = bract; *l*<sup>2</sup> = second pair of leaves; *l*<sup>3</sup> = third pair; *ax*<sup>2</sup> = axis of axillary bud; *ax*<sup>1</sup> = primary axis of cone.

beneath the scale, and for this latter reason only, it could not be a foliar organ, because *folium in axillâ folii* is a thing without example in the whole vegetable kingdom.'

Von Mohl (38) in 1845, in a paper dealing chiefly with the male organs of Coniferae, makes the interesting statement that in *Pinus*, &c., the bract of the female cone is greatly reduced and a leaf of a secondary axis replaces it. The same thing occurs in the vegetative shoot, where the foliage-leaf belongs to a secondary shoot and the subtending leaf is



reduced to a scale. He says also that in *Thuja*, *Cupressus*, *Juniperus*, the scales bearing the *ovules* are modified leaves of the *primary* axis, as there is no sign of any other bract, and in the vegetative shoot there is a gradual modification of the leaves from the seedling upwards.

Alexander Braun, the renowned German botanist, must take credit for being the first, viz. in 1853, to promulgate the view that the seminiferous scale consists of the two first leaves of a *bud axillary to the bract*, these leaves being fused by their margins (Fig. 3).

Baillon (54, 62), on three separate occasions, in important papers, advocates the ovarian theory of the ovule, making a determined stand against the opposite theory of Gymnospermy.

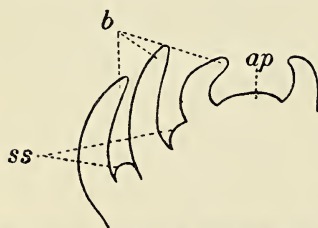


Fig. 4. Diagram of part of cone of Abietineae, showing the origin of the seminiferous scale (*ss*) in the axil of the bract (*b*); *ap* = apex of axis of cone. (After Ørsted.)

He bases the chief weight of his argument on the *development* of the organs concerned; he observes the seminiferous scale appearing as a papilla in the axil of the bract, just like the bud of an incipient branch (Fig. 4), and concludes therefrom that it must be of axial nature, forgetting the fact that structures may, even in their youngest stages, be already

congenitally modified, i.e. inheriting directly, without passing through the previous progressive stages, the adapted structure of the same organs in their parents. His firm conviction that the integument of the ovule is an ovarian wall is based chiefly on the fact that, in the earliest stage of development, that structure appears as two distinct papillae, which, as he thinks, must be the two carpels composing the ovary. But one fails to see exactly why this should be the interpretation, for there is variation in this respect among plants. Assuming the ovular envelope in the genus *Podocarpus* to be an ovary, we find that in one species, *P. chinensis*, Sw., it arises as a continuous ring, but in another species, *P. dactydioides*, A. Rich.,



as two papillae, so that there is just as much reason in this case for considering it an integument. The following are his main conclusions:—'The female flower is either terminal or placed in the axil of a bract or a leaf; but it is always borne by an axis and never by a bract. But the form of the axis is very variable, as is characteristic of receptacular organs. The flower, as Mirbel and Spach also thought, is not gymnospermous, but possesses an ovary of two carpels, without floral envelopes, containing an orthotropous and erect ovule on a basilar placenta. The cupule, of variable consistence and shape, surrounding this ovary, which in several genera has received the name of aril, is a late production, although it arises previous to fertilization, and is of the nature of a disk.'

Baillon will always be regarded as the champion of the developmental theory.

Caspary (53) maintained, with Braun, from the fact that he found buds in the axils of the bracts, having the two halves of the seminiferous scale as lateral appendages, that the seminiferous scale represented, in reality, the two first leaves of a vegetative bud, sometimes called 'the cotyledons of the branch' (Fig. 3), which were fused by their *anterior* or external margins. Of this view of the matter there will be more to say further on.

In 1864 the Italian botanist, Parlatore (60), gave a valuable and interesting contribution to our knowledge of the structure of the female organs in various genera. He considers the seminiferous scale to represent a shortened axis in the axil of the bract with fused and broadened leaves. The fact that it is often bifid, emarginate, notched, &c., proves its compound nature. The seminiferous scale represents the leaves of the axillary axis, which is limited to a single point at the base of the scale. In a cone of *Pinus Lemoniana* in the Chiswick Gardens, he observed the ordinary short shoots or brachyblasts, each with its two needles, springing from the axil of every alternate bract in place of the seminiferous scale. He upholds throughout the ovarian theory; the ovule being for

him always a 'flower.' In the Taxineae and Podocarpeae the 'aril' is composed, in the first case of one, in the second of two bracts of the axillary axis, which here become fleshy, and are homologues of the seminiferous scale.

In the same year (1864), Ørsted (61), a Danish botanist, produced a paper in which he describes and figures some highly interesting and instructive abnormal cones. He mentions three cases of cones, in the lower part of which the bracts had the form of foliage leaves, while higher up they assumed that of ordinary bracts, in the axil of each of which were several scales arranged as on a suppressed shoot, the two outermost of which were the largest and placed opposite each other. Still higher up, the bract gradually became reduced in size, as did the scales in number; but the outermost scales gradually increased in size, at the same time becoming connate by their margins, while rudimentary ovules appeared at the base on their dorsal side. At length the bract became reduced to its ordinary minute size, and the seminiferous scales fused into a single large broad structure, dentate or bifid at the apex. From these facts he draws the following conclusions:—

(1) 'The cone arises from the metamorphosis of a shoot, as the leafy bracts become changed into the bracts of the cone and the buds into seminiferous scales. (2) The seminiferous scales arise, like any other flower, by metamorphosis of a bud, but only two leaves of the flower are formed, viz. the two seminiferous scales which fuse together (Fig. 3). (3) That the seminiferous scales in Abietineae are open carpels with naked seeds is seen by the fact that this metamorphosis agrees entirely with misformed carpels in other plants (e.g. *Paeonia Moutan*). (4) That a leaf may represent a whole bud, and as such may be placed in the axil of another leaf' (Fig. 1). In the Larch, a malformation in just the opposite direction occurred, in which the cone was normal in structure at the base and proliferated at the top, and there resembling a vegetative shoot. The seminiferous scale was at the base at first quite normal, higher up becoming bifid; then split

into two distinct leaves; and finally representing two scales enclosing a small bud.

In *Picea alba* a cone had proliferated into a short vegetative shoot bearing a few leaves and a bud. There was an abrupt transition between this and the normal part of the cone below. The terminal bud eventually developed into a leafy branch.

From the study of the development of a cone of *Pinus montana* var. *Pumilio*, he draws the following conclusions:—  
'(1) That the seminiferous scale arises in the axil of the bract just like a bud (Fig. 4), and can aptly be compared with a young bud and bract in *Bryonia dioica*, so that the development agrees with what was seen in the monstrosity. (2) The organs considered by R. Brown as ovules agree in their early origin and further development with the ovules of flowering plants in general. (3) The peculiar form of the seminiferous scale in the Pine cannot be properly understood without taking into account its growth, which extends over several years, and, after a period of rest, is once more renewed, this being connected with the slow process of fertilization.'

He then proceeds to describe the development of the cone-scale in Cupressineae and Taxodineae. At the end of the paper he draws the following general conclusions:—

'I. Both the normal development of the flowers and the various sports strengthen R. Brown's observation that the ovules are naked.

'II. The cone-scale in Abietineae is an open carpel, arising as a bud, and representing a flower. It consists (at least in *Picea* and *Larix*) of a fusion of two leaves, corresponding to the young scale-leaves of the ordinary bud. The notching at the apex of the scale is an indication of this fusion.

'III. The cone-scales in Cupressineae correspond to the bracts in Abietineae. They only rarely preserve their original form (in *Actinostrobus*); they usually undergo a transformation which does not result from a fusion of bract and carpel, but is the same change which occurs when a leaf becomes peltate. The cone-scale consists, therefore, of a single leaf.



Only in the Cupressineae verae is the scale completely peltate, in Actinostrobeae and Thuiopsidae it is incompletely so. The Cupressineae have no carpel, but the flower is represented by  $12-\infty$  naked ovules, always at first attached to the axis of the cone in the axil of each scale, but which, later on, as seeds, become removed and carried up by the stalk of the scale, and in this way come to be situated on the latter.'

The name of Sachs (82), through his classical 'Lehrbuch,' first published in 1868, lends great weight to a view, then first enunciated, that the seminiferous scale in the Pinace (*Abies*, *Picea*, *Larix*, *Cedrus*, *Pinus*) is really a ventral outgrowth of the subtending 'bract,' of *ligular* nature, such a structure, in fact, as we are familiar with in *Isoëtes* or *Selaginella*; so that there is in reality but one single scale instead of two, and the 'bract' is part and parcel of the carpel, bearing the ligular placenta, with inverted orientation of its vascular bundles. 'In the other Abietineae,' the seminiferous scales 'do not spring from the axils of leaves, but grow immediately out of the axis of the cone, and are therefore themselves leaves and of a carpellary nature.' This view will be further developed hereafter.

Van Tieghem (65), in the following year (1869), assumed a prominent and important rôle in the endeavour to solve the problem, by following a line of investigation different from all which had preceded him, viz. that of the evidence from the *anatomical structure*. As Baillon is the champion of the developmental method, in the same way must Van Tieghem be regarded as the champion of the anatomical method of research. His primary idea is that the seminiferous scale in all Coniferae represents the first and only leaf of an axillary branch, though he suggests the possibility of its being really two fused leaves. His view is based on the course and orientation of the vascular bundles. In the Abietineae he finds that the bundles of the bracts and seminiferous scale, as they respectively leave the central cylinder of the axis of the cone, are enclosed each in its own parenchymatous sheath, and thus represent independent



systems of bundles. The uppermost bundles divide up to form an arc with inverted orientation, i.e. with their xylem directed outwards and downwards; it is this arc which is the system belonging to the seminiferous scale, while the lower bundle, with normal orientation, belongs to the bract. One of the end-bundles of the arc sends off a strand on either side to the ovules. 'The arrangement of the bundles in an arc shows that the axillary organ is a leaf and not a branch; the outward orientation of this arc shows that this leaf is placed diametrically opposite the bract; its origin shows that this leaf belongs to the axillary branch of which it is the sole representative, i.e. of which it is the first and only appendage. The first leaf bears the ovules on its dorsal surface.

'In *Sequoia*, *Athrotaxis*, and probably also *Sciadopitys*, the foliar bundle and the upper bundles remain enclosed in the same parenchymatous sheath from the point of their insertion on the axis until near the apex, then the parenchyma separates, the two organs become isolated, and whilst the bract ends in a "languette," the first leaf of the bud continues its development, growing a short way beyond the bract. The scale is thus double.

'In the Cupressineae, *Taxodium* and *Cryptomeria*, the same structure obtains, except that the base of the seminiferous scale between the ovules and the axis is not elongated at all, and thus the ovules are situated at the very base of the scale.

'In *Araucarieae*, elongation only occurs below the insertion of the ovules. In these plants the scale is double, as in *Sequoia*, &c., the two sets of vascular strands being included in the same parenchymatous tissue until beyond the point of insertion of the ovules. In *Araucaria brasiliana* there are four bundles in the seminiferous scale, each set of two widely separated and orientated sideways with their xylem directed inwards; opposite the median portion of the ovule they turn outwards, and are often *concentric* in structure, with the phloem occupying the centre. In order to form the ovule, the seminiferous scale becomes reflexed towards the *ventral*

side, as is shown by the fact that its bundles enter the ovule on the upper side of the latter; this fact will also explain the contact of the ovule with the bract and the parenchyma which surrounds it on its upper side.

‘In *Dammara* the ovule has become reflexed towards the dorsal side.

‘Finally, it may happen that the upper bundles, instead of developing, as in the three preceding cases, sometimes both above and below the ovules, sometimes only above, or only below, are scarcely prolonged either above or below. Then the leaf is terminated by the ovule or ovules, which arise from the transformation of its entire lamina, and this leaf is sessile, or at least its petiole is exceedingly short; the ovule represents in itself the first leaf of the axillary branch almost entirely, in other words, the carpel, while retaining in its vascular system the origin, orientation, and structure proper to it, is reduced to its ovular portion,’ as in *Podocarpus*, where the first leaf of the branch is folded on its dorsal surface to form an anatropous ovule, and in *Phyllocladus* and *Pherosphaera*, and some species of *Dacrydium*, where the leaf having remained straight, produces an erect, orthotropous ovule. In these plants the ovular leaf is completely independent of the bract. In other species of *Dacrydium*, *Saxegothea*, and *Microcachrys*, the bundles of the bract and ovular leaf are contained within the same parenchymatous sheath, for a third or half of its length, as a result of which the ovule is only partially inverted. In the Taxineae the two upper bundles, which in the other groups supply the seminiferous scale, here divide up to form a circle or cylinder, and pass into a developed axillary shoot of secondary order bearing several bracts, some of which, or only one, bear in their axil a shoot of the third order, which either is only represented by its first leaf, or forms bracts, in the axil of some of which axillary shoots of the fourth order are developed. In *Cephalotaxus* the female bud (primary shoot) forms in the axil of a leaf a shoot of secondary order bearing three or four pairs of bracts, all of which are usually fertile. Each of these

receives its own bundle, and two upper bundles which become inverted, and pass each to one of the two ovules, which represent the bilobed first leaf of the axillary shoot. In *Taxus*, the female bud (primary shoot) forms in the axil of one of its leaves a secondary shoot bearing three pairs of bracts, of which the sixth is alone fertile. These bracts are arranged with a divergence of one-fourth or one-half, and are falsely decussate and arranged as if for the commencement of a two-fifths phyllotaxis. Two bundles leave the axis at the sixth and last bract, and both, becoming inversely orientated, enter the ovule, which represents the first leaf of an axillary shoot. The origin, orientation, and structure of its vascular system show that this is the true interpretation. The two opposite bundles of the wall of the ovule are *concentric*, the phloem occupying the centre.'

In *Torreya* the structure is the same, except that there are a greater number of fertile bracts, and that there is *one further stage of branching* than in *Taxus*.

In the same year Sperk (66) issued an important work on the subject of Gymnospermy. He found a hermaphrodite cone of *Larix* with male organs at the base and female at the top. He regards the ovules as ovaries. The seminiferous scale is of foliar nature. In *Cunninghamia*, sometimes the ovule, sometimes the seminiferous scale is formed first; in a proliferated cone the bract had three distinct leaves in its axil, which were the three seminiferous scales; between these latter and the bract was a long stalk thickened upwards, which perhaps represented a secondary axis. In *Taxus parvifolia* the outer ovular envelope is a continuation of the whorl of bracts below the ovule; the carpels are at first separate and distinct in a whorl, and later, by pressure of the bracts, become fused into a whole. In *T. baccata* the integument arises *from the axis*, and leaves a space between it and the nucellus. In *Cupressus lusitanica* the integuments were proliferated into leaves. In considering the case of *Cryptomeria japonica* he gives the following reasons why the inner scale cannot be a carpel:—'(1) In young cones the ovary



arises immediately in the axil of the bracts, as in other Cupressineae; later on small scale-like outgrowths appear between the ovaries and the bracts, which cannot be carpels as they appear later than the so-called ovules. (2) The ovaries are often not placed in the axils of these inner scales, but alternate with them, and are lateral. (3) The number of the ovaries is independent of the number of these scales; often more scales occur than ovaries, or vice versa. (4) In the anomalous development of the scale it may fuse with the ovary and increase the size of the latter. (5) In anomalous development of ovaries their foliar nature becomes apparent.'

He considers that abnormalities are of importance and follow a definite law.

In *Juniperus Hartwissiana* he finds certain objections to the carpellary theory of the bract. (1) There are an indefinite number of ovaries at the base of the bracts, three, five, or four; in the last case two ovaries occur in the axils of each two bracts, while the third is left empty; which shows that the presence of ovaries is not a necessary character of the bracts. (2) The ovaries often alternate with the bracts, as if continuing the whorl of three leaves upwards. The ovary first arises as four small protuberances.

These are his concluding remarks:—'(1) The development of the ovule of the so-called Gymnosperms corresponds entirely with that of the ovary, and not the ovule, of other Phanerogams, viz., (a) the so-called integument arises earlier than the nucellus; (b) as a result of this, the former arises from the receptacle and not from the nucellus, as a true integument should do; (c) it is formed of distinct carpel-leaves, which fuse sooner or later, a fact which does not occur in any integument. (2) The so-called integument arises independently, without a nucellus, and remains long separated therefrom, surrounding it loosely, and leaving often a considerable space between. (3) The simple structure of the ovary of Gymnosperms is no reason for considering the latter as an ovule. (4) The anatomical structure of the so-called integument is too complex for such an organ. (5) The



formation of a style and stigma in some Coniferae can only point to the presence of an ovary. (6) Several anomalous structures exhibit the foliar nature of the ovary. (7) The structure, form, and development of the ovary are repeated in the Loranthaceae, Amentaceae, &c. (8) The idea of R. Brown and others that the scale surrounding the flowers is an open carpel, is refuted by all my observations and investigations.'

Sperk does not himself attempt, however, to give any explanation of the seminiferous scale.

In the *Botanische Zeitung* for 1871, Von Mohl (69) first gave to the world his very interesting and important discovery concerning the 'double needle' of the Umbrella Pine, *Sciadopitys verticillata*. He showed that its origin was the same precisely as that of the seminiferous scale of the Abietineae, viz. from the two first leaves of a secondary axillary shoot, which have become fused by their inner or posterior margins; as a result of which the *ventral surface of the organ is directed outwards* (Figs. 1 and 3). The small axillary shoots or brachyblasts of the female cone of Abietineae and those of the vegetative shoot of *Sciadopitys*, abnormal in the one, but normal in the other, are therefore homologous structures, and, by the aid of the former abnormality, the eccentric orientation and structure of the 'double needle' is readily explained, though it required the acute insight of a Von Mohl to solve the riddle.

Strasburger's (70 and 85<sup>a</sup>) view of the matter, expressed in his renowned work on the Coniferae and Gnetaceae, in 1872, is sufficiently original, not to say, at least for some of us, bizarre, and approaches more nearly to the views held by Baillon and by Schleiden, than to those of any other botanists. In the first place he maintains the ovarian theory of the ovule, a view which he afterwards abandoned in favour of Gymnospermy in his later book on Angiosperms and Gymnosperms. But the view as to the nature of the seminiferous scale knew with him no variation, at least in essentials, from beginning to end. He held this organ in the Abietineae

to be of the nature of a flattened axis bearing rudimentary leaves, or more definitely stated, a *disk*, chiefly on account of its late development compared with that of the ovules. Confronted by the abnormal appearance of the axillary shoot in the axil of the bract, accompanied by the splitting up of the seminiferous scale into two parts, which became transversely seated on the axillary shoot as its two first leaves, he regarded this metamorphosis of the seminiferous scale merely as the result of the struggle waged between two opposing forces, viz. the vegetative development of the cone and the normal formation of reproductive organs, in which the former had for the time being overbalanced the latter. The fleshy, outer envelope of *Taxus*, &c., he also regarded as possessing a discoid character.

Next are to be mentioned some most striking abnormalities in cones of *Picea excelsa*, Link, observed by both Stenzel (84) and Willkomm (86). The former, in 1876, described a cone in which in the axil of the bract a leafy bud arose, whose first two leaves were harder and browner and more erect than those of the ordinary vegetative shoot, and resembled more the seminiferous scale; they were directed somewhat towards the axis; the following pair of leaves were median, anterior and posterior (Fig. 3). No ovules were to be seen. The pair of larger first leaves were often fused with the small leaves of the bud. He concludes that 'the seminiferous scale of the Spruces consists of the first two leaves of an otherwise undeveloped branch arising in the axil of the bract, these leaves being fused by their posterior margins, and *thus having their dorsal side directed towards the axis of the cone*, and bearing each on this side an ovule. When the seminiferous scales fuse by their *anterior* margins, it is to be considered as an exceptional case, like that of Ferns with sori on the upper surface of the fronds.'

On another occasion he found, in the same plant, androgynous cones, in which the male organs usually occupied the base and the female the upper part; more rarely were the male scattered amongst the female; and still more rarely

did the male form a middle zone with female above and below. Some of the bracts bore pollen-sacs. He also possessed at this time a proliferated cone of *Picea alba*. The buds in the axils of the bracts bore, besides the two seminiferous scales fused by their posterior and gaping at their anterior margins, a posterior and an anterior scale, and one or two inner scales. In some cases the seminiferous scale was so completely fused with the anterior bud-scale as to form a single flat scale as seen from the front, but in reality its posterior margins were represented by two low ridges, visible from the inside, which did not, as in other cases, extend as far as the posterior bud-scale. As regards the characteristic projection or 'Dorn' on the seminiferous scale of *Pinus*, which Strasburger thinks is an *axis*, it may represent either the place of fusion of the posterior margins of the seminiferous scale, or the posterior bud-scale.

The same kind of sports as those in *Picea* occur also in *Tsuga Brunoniana*, Carr., a fact which, he says, lends weight to the above explanations. In the latter plant, the posterior bud-scale is often as well developed as the anterior one, so that the parts of the bud all come to be united laterally into a woody structure. The axis of the bud is often more elongated into a leafy shoot. The fact that the seminiferous scale is shown to consist of two leaves, is antagonistic to the idea that the ovule is an ovary. The fact that the ovular envelope arises at first as two distinct outgrowths which afterwards become fused, does not prove that they are part of an ovary; and, moreover, there is no stigma present.

Willkomm (86) observed in a proliferated cone of the Spruce great numbers of *buds* in the axils of the bracts of the upper portion, which differ from those in Stenzel's case in being extremely symmetrical and regular, and therefore hardly to be called 'monstrosities,' such as those of Stenzel; this is a very important factor in determining their morphological value. His conclusions as to the morphology of the seminiferous scale are precisely the same as those of Stenzel. The monstrous tubular or funnel-shaped anterior scale of



Stenzel's buds never occurs, this scale being always quite regular in shape. The posterior bud-scale is always quite rudimentary. The explanation lay probably in the fact that Stenzel's cones were, as a whole, more monstrous, while his own possessed exceptional symmetry and regularity. He believes that the seminiferous scale proceeds from an axillary shoot with two opposite leaves and bearing a terminal bud (Fig. 3). 'It is therefore a *metamorphosed brachyblast*, consisting of a *median axis and two leaves fused therewith on its anterior surface, whose original separation in P. excelsa is shown by the indentation of the apex of the seminiferous scale.*' He regards the seminiferous scale as consisting of 'two open carpels, and the ovules or seeds occurring on their dorsal side are naked.'

Although Čelakovský produced at this time, and a few years later, some literature on the subject, I prefer to treat his views as a whole later on, for it was not until 1897 that his maturest ideas on the whole problem were issued to the world.

Arcangeli's (90) views, published in 1880, are perhaps worth mention on account of their extraordinary nature. He considers, from the fact that the two sets of bundle-systems arise as one from the axis<sup>1</sup>, that the bract and seminiferous scale in *Pinus*, *Cupressus*, *Thuja*, *Cryptomeria*, and *Sequoia* are really a single organ, of *axial* nature, the bract being a leaf of this branch, and the latter arising in the place of a leaf. In *Cunninghamia* 'the scale is developed chiefly with the characters of a leaf, since there is only the small upper bundle to indicate its rameal character.' In *Araucaria* the scales are true *bracts* showing no sign of a transformation into shoots, for the reason that the bundles show no sign of arranging themselves in a vascular ring, the two laterally-placed bundles which Van Tieghem considered as belonging to the upper scale being only branches passing off from the bract-bundles to the ovules. 'One must admit that the

<sup>1</sup> But this is, however, by no means always the case. See my former paper: 'Observations on the Vascular System of the Female "Flowers" of Coniferae.' Ann. Bot. Vol. xiii, 1899.



appendages of the cones of Coniferae may vary as regards their morphological significance,' so that the ovules may be inserted either on a leaf or a shoot. These strange branches he compares to the 'cladophylls' of *Lycopodium*, as in *L. Selago*. He rises superior to the difficulty of branches arising on the stem in place of leaves by assuming that *the leaf belongs both to the stem and to the branch in its axil*. For the axillary branch is part of the *same organ* as the leaf. He regards the ovule and pistil as being both of axial nature. The seminiferous scale, although a modified branch, is nevertheless the representative of the 'carpellary involucre' of other plants. He believes that Conifers, like Cycads and Gnetaceae, are Gymnosperms. All this appears to me, however, a rather irrational view of the whole matter.

The theory which to-day receives, perhaps, as much support from botanists as any other is that first put forward by Sachs, as we have seen, in 1868, and afterwards in 1881-2 more elaborately worked up and more definitely stated by Eichler (71, 91, 95). This theory maintains that there is but one scale, and no double scale, in Coniferae, representing a carpel or sporophyll bearing an ovule or ovules on its upper surface or on its axil; and that the so-called seminiferous scale is in all cases of the nature of a ventral outgrowth of the scale, representing a placenta or ligule, and therefore forming an integral part of the scale (Fig. 2).

In *Dammara* the bundle given off to the ovule is nothing more than an ordinary bundle leaving the carpel for the ovule: it is absent in the sterile scales, a fact which seems to show the absence of an inner scale. As regards the inverted orientation of the bundle going off to the ovule, he says: 'In all cases, where a leaf forms superficial products, which have to be provided with bundles, these last twist their elements round.' He compares other outgrowths of leaves, especially the fertile spike of *Ophioglossum*, with the ovule of *Dammara*; in all such cases the parts of the bundle are inverted. The scale is a single leaf and its appendage an ovule. It cannot be an ovary as Baillon, Parlature, Dickson, and Sperk say,

because an ovary is the prolongation of an axis, and cannot occur on a leaf. In *Araucaria* the scale receives a *single* bundle which divides up laterally and a little above the base, sending off a few small branches to the ovule (Fig. 2), which have inverted orientation as in *Dammara*. The development, as far as is known, agrees with that of *Dammara*. The outgrowth above the ovule (Fig. 2) is a *ligular* structure, like that in *Isoëtes*, and the velum of the latter is analogous to the integument of *Araucaria*. The inner bundle-system is absent in sterile scales, as in *Dammara*. In *Cunninghamia* a single bundle enters the scale. Three bundles pass off, with inverted orientation, one to each ovule. Thus he considers that no inner scale can be present, and there is but a single leaf, and the transverse ridge is a ligule. In *Sciadopitys* there is also a single leaf with a ventral excrescence. In the Abietineae, the bract appears first in the development, then the seminiferous scale as a transverse swelling on its inner surface (Fig. 4). The bract receives a single bundle which does not further divide up. One or two bundles enter the seminiferous scale which, according to the form of the basal part of the scale, are either given off from the bract-bundle or directly from the axis of the cone; the bundle, on entering the seminiferous scale, divides up into several with inverted orientation. The seminiferous scale is thus an inner outgrowth of the bract, both together constituting a single leaf. Many think the two are quite distinct. But where they leave the axis, though only for a short distance, they are united, and the development shows one to be an outgrowth of the other. Schleiden's and Strasburger's idea that the seminiferous scale is a flattened axis or cladode, does not agree with the arrangement of the bundles, for in all cladodes the xylem is directed inwards, the bundles being either flattened together or grouped around a centre. As an objection to the views both of Van Tieghem and Von Mohl, he says that there is nothing to be seen of an axillary bud, but the seminiferous scale appears as an inner outgrowth of the bract. As an objection to Mohl's view, there is no sign of the seminiferous scale being formed of two

leaves, as, from the earliest stage onward, it appears as a single outgrowth. Neither do there occur two symmetrically-placed bundles, one belonging to each part of the scale, as in the needle of *Sciadopitys*, but, on the contrary, the single one, or the two in the base, divide up into a number, all having the same orientation. Owing to the abnormal appearance of an axillary bud and its great pressure on the seminiferous scale as a result of the compact arrangement of the scales on the cone, the bud causes the separation of the seminiferous scale into two parts, whereby it assumes the appearance of bearing them as its two first leaves.

In the Taxodineae there is a distinct ventral outgrowth, with its own bundle-system, whose parts have inverted orientation. In Cupressineae the ovules are so close to the base of the bract that they may be called axillary. Where many ovules occur in the axil they are seated on the axis of the cone for a short distance as well as on the scale (*C. semper-virens*), so that no strict distinction can be made between the ovules which are developed on the axis and those arising on the scale. They usually occupy the middle of the axil. Where two are present they occur right and left; in *Juniperus* one of these is usually absent. No differentiated ventral outgrowth is present in this order; the scale appears either quite simple or exhibits a more or less pronounced ventral swelling depressing the bract, as in *Sequoia* and *Taxodium*, and more or less clearly distinguished from it. The inverted orientation of the upper bundles is caused by the peltate thickening of the scale, and, as a natural consequence of this, the scale is at first exactly similar to the lower vegetative leaves; in the base of the scale all the bundles are arranged in a circle just as in the stalk of any peltate foliage-leaf. Although the scales cannot be said with certainty to bear the ovules, and possibly represent only bracts, they are in reality open carpels. In the Podocarpeae there is also but a single leaf. In *Microcachrys* the inner bundle-system does not even exist, which is due to the fleshiness of the carpel. It is represented in *Dacrydium* by two weak branches, with inverted



orientation. Both plants have an outer integument in the form of an arillus. In *Podocarpus dacrydioides* the ovule is inverted and fused along its whole length with the carpel. The second integument becomes fleshy like an aril, and arises later than the first integument. Just above the base of the carpel the bundle gives off two branches which terminate just above the nucellus of the ovule. The inner bundles become inverted because they arise from the inner surface of the carpel. In the remaining sections of *Podocarpus* the ovule is deeply divided from the carpel, which is overtopped by the anatropous ovule. Here the inner bundle runs up the raphe, where it divides up into several branches which bend back above the nucellus, forming a circle around it, with the xylem-ports all directed outwards. Strasburger and Van Tieghem say the 'raphe' is the inner scale, but it is not so; there are other cases in Angiosperms where the raphe is thus strongly developed. In the section *Nageia* and in several species of the section *Eupodocarpus*, the male flowers occur in a capitate inflorescence in the same position as the receptacle with ovules, so that each ovule really represents a flower subtended by a bract, and the whole receptacle is not a single flower. But in *P. Sellowii*, *P. salicifolia*, *P. Thunbergii*, &c., the receptacle corresponds to a single male flower, so that the aggregation of distinct and unfused carpels in *P. spicata* is to be considered as a single flower. In *Phyllocladus* the ovule is axillary and not borne on a carpel enclosed in an aril; the two bundles supplying it have their xylem turned towards *each other*, not towards the carpel. In *Cephalotaxus* the bundle-system is the same as in *Phyllocladus*. In *Taxus* and *Torreya* the ovular axis bearing the bracts receives two bundles with their xylems directed towards each other. An aril is present. Seeing that in these last two genera the ovules are borne on independent leafy shoots, the whole cannot be called a flower, but each ovular axis is such. The ovule is clearly of axial origin. Eichler compares the different positions of the ovule with the similar variations in position of the macrosporangium as in *Isoëtes* (where it occurs on a leaf), *Selaginella* and *Lycopodium*



(in the axil), *Psilotum* and *Tmesipteris* (at the end of a short branch, and thus axial), all of which plants belong to the same circle of affinities to which Conifers are most nearly allied. The ovule is not a bud or leaf-segment, but a structure *sui generis*. A terminal ovule occurs in *Polygonum*: terminal and axillary ovules in Piperaceae, Balanophoreae, &c. Where an ovule arises from a leaf, the latter is a carpel. Seeing that in Taxodineae the leaves which function as ovule-bearing carpels are homologous with those in Cupressineae which have ovules in their axil, it follows that the latter are also carpels. The envelope of the nucellus in *Taxus* and *Torreya* is an integument, as shown by the position of the arillus in other genera in which ovules are borne on a leaf. The Araucarieae, the oldest Coniferous type, are nearly allied to Cycads. In agreement with the palaeontological sequence, the ovule in Cupressineae and certain Taxineae (the most recent types of Conifers) becomes axillary, until in *Taxus* and *Torreya* it becomes an independent flower, borne on a leafy stalk arising from the axil of a leaf.

Cycads, Coniferae, and Gnetaceae are all gymnospermous. In the latter the outermost covering of the female flower is to be considered an ovary, not quite closed and without stigmas. In *Juniperus* the carpels are closed, but there are no stigmas, and the pollen reaches the ovule directly, so that the latter is therefore gymnospermous.

The Sachs-Eichler theory of this difficult subject no doubt appeals to many owing to its great simplicity, deriving as it does the solution of the problem directly from the structures as they are presented to us to-day; its tranquillity of contemplation disturbed neither by the idea of a possible adaptive modification of these structures from others once totally different in appearance, nor by the alteration which they undergo in cases of the abnormal metamorphosis of parts: indeed, towards the latter Eichler is actively hostile, for he says—and this summarizes his general attitude in the matter—'I cannot forbear showing how forcibly the present case once more warns us that monstrosities cannot be brought to bear

on the examination of normal relations. Without those abnormalities, no one would have thought of regarding the seminiferous scale of the Abietineae as a composite organ, and we should then have been spared, or at least partially so, the complicated theories which have for so long obscured the understanding of the floral structure of one of the most important groups of plants.' Whether or not this is a sound and safe position to take up, will be seen when the most recent view of this matter is brought forward. However, both the developmental and the anatomical evidence favour it.

Velenovský (101), in 1888, described an interesting case of an abnormal Larch-cone. One axillary bud *bore ovules on the lower surface of all its leaves*. When the bud is suppressed, the two seminiferous scales *orientate themselves with regard to the axis of the cone*. In some tropical climbing *Aristolochias*, the first bract of a suppressed bud, which it covers over when young, at length increases in size and becomes *orientated with regard to the stem* which it clasps as an ochrea. The same thing was observed to occur in *Tilia grandifolia*, where the bud in the axil of the flower-bract was suppressed, so that the bract was orientated with regard to the axis in the same plane as the subtending bract, and with its upper surface directed to the latter. The double scale of a cone of Abietineae is a kind of brachyblast, and is the same morphological phenomenon as occurs in *Sciadopitys*, so that it is nothing new for this type of plant. His concluding remarks are worthy of special notice:—'The deformed cones of our *Larix* and also the Spruce-cones of Caspary, Stenzel, Willkomm, and Čelakovský, are not monstrosities in which certain parts are irregularly and haphazardly developed. We find in all stages of the scale-metamorphosis a definite law and the greatest regularity of development, so that, by retaining a right grasp of the process of development, we can *a priori* expect and, as a matter of fact, shall find, the distinct developmental forms. Such a regularity, which occurs in every normal flower, can never be an equivocal, pathological and casual phenomenon.'

Masters' (105) position was in 1884 that of Eichler, viz. that the seminiferous scale is an excrescence of the bract; this is stated in a paper on the comparative morphology of *Sciadopitys*. In this paper he makes the statement that 'the adult seed-scale of *Sciadopitys* and of Abietineae occupies the same apparent position with regard to the bract that the "needle" of *Sciadopitys* and the fascicle of "needles" of *Pinus* (with its sheath) do respectively to the true leaf.' From the consideration of an abnormality in which the 'needle' was deeply forked and from the fork sprung a short axis bearing a whorl of 'needles,' he thinks the 'needle' is of axial not of foliar nature. But this phenomenon, it seems to me, might also be interpreted as the result of the *elongation* of the otherwise suppressed or shortened axis of the brachyblast bearing the two leaves fused by their posterior margins, causing a separation of the latter, while the axis remained for part of its length *fused with one of its two leaves*, and after becoming free higher up, produced fresh leaves. In his paper of 1891, he puts forward a view of the seminiferous scale which, amongst all those which occupy our attention, has at least the distinction of being unique. I give his own words:—'Reverting to Casimir de Candolle's "Théorie de la Feuille" . . . this botanist compares the leaf to an axis, the *upper half* of whose vascular system is abortive or undeveloped, for which reason the xylem is towards the upper or inner surface, the phloem towards the lower. Apply a similar explanation to the fruit-scale, and the position of xylem and phloem becomes intelligible. According to this view the fruit-scale is an enation, either from the bract or from the axis, it is immaterial which, of the nature of a cladode or modified shoot. The *lower or outer* portion of this branch or cladode is abortive, and consequently the xylem is towards the lower or outer, the phloem towards the upper or inner surface' (Fig. 5). This view, however, appears so incongruous and incomprehensible, and offers for our contemplation a structure so anomalous, and so foreign to all other divisions of the



vegetable world, that it is not likely to find favour with many botanists.

Penzig (109), in his 'Pflanzen-Teratologie' of 1894, supports, with great enthusiasm, the position of Delpino. This latter author considers the carpels of Conifers as consisting of three parts, of which the middle part forms the bract, while the two lateral lobes bend inwards and fuse together by their margins to form the seminiferous scale, bearing the seeds (Fig. 6). When an axillary shoot appears, it separates

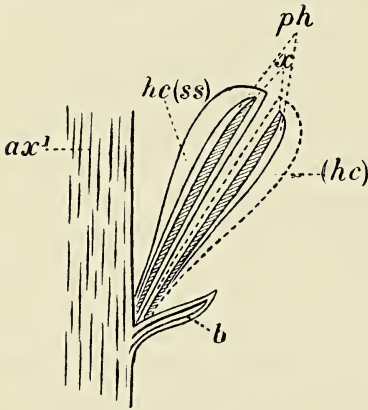


Fig. 5. Diagram showing the seminiferous scale *hc(ss)* and the hypothetical missing half of the cladode (*hc*); *b* = bract; *ph* = phloem; *x* = xylem; *ax¹* = primary axis of cone.

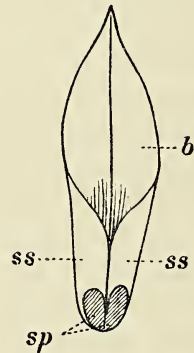


Fig. 6. Diagram of bract, showing its two basal lobes involuted and fused together to form the seminiferous scale (*ss*); *b* = bract; *sp* = sporangia.

the two fused lateral lobes of the carpel, which bend back again and assume their former lateral position. When the shoot arises at the same level as, or between, the seminiferous scale and the bract, the two lobes come to have their ventral surfaces directed towards each other. But when the axillary shoot arises *between the seminiferous scale and the axis of the cone*, the two placental lobes have their dorsal sides directed towards the axillary shoot; a fact which, says Penzig, is not explicable on the Braun theory (that the scale represents the two first leaves of the axillary shoot), but is quite easily



so on that of Delpino. That the lobes, as the metamorphosis proceeds, become more leaf-like, is only in accord with their foliar nature. When, by elongation of the axillary shoot, the two lobes are carried up by it, it appears as if they were its own appendages; but they are in reality, like the bract, members of the axis of the cone, receiving their bundles directly from it. The two medianly-placed scales of the axillary shoot are to be looked upon as its first-formed leaves, their antero-posterior position being explainable from the fact that they could not occupy the normal transverse position, for this is taken up by the placental lobes. He (Penzig) thinks the phenomenon observed by Velenovský in a Larch-cone of an axillary shoot bearing, besides the two fleshy placental lobes, five other fleshy ovule-bearing scales, speaks strongly in favour of Braun's theory. But he has previously shown how *a specific metamorphosis may be determined by adjoining organs*. In *Antirrhinum* the anterior stamens adjoining the petals in double flowers often acquire the form of the lobes of the lower lip, while the upper stamens acquire the form of the upper lip of the corolla; the two posterior stamens in Orchids, when they are actually present, acquire the form and colour of the labellum because they adjoin the latter. He attributes these cases to a wandering of the specific structural substances into the neighbouring organs. In the same way he explains the above case of the Larch; the special substances intended for the placental lobes have wandered into the axillary shoot and influenced its appendages in the same way as the lobes. The transformation of foliage-leaves into sexual organs is not uncommon in *Coniferae*.

Delpino's theory also enables us to understand how (as in *Araucaria brasiliensis*, A. Rich, *Picea excelsa*, Link, *Tsuga canadensis*, Carr.), the seminiferous scale splits into two lobes without the appearance of an axillary shoot.

Noll (1908) in 1894 examined proliferated cones of the Larch, and found the most gradual and continuous series of transitional forms between the seminiferous scale and an

axillary bud, so as to leave no doubt in his mind as to the morphological equivalence of the seminiferous scale and the two first leaves of an axillary bud. In one point his series was better than in other previously described cases: the axillary bud occupied a position *anterior to the seminiferous scale or its two component parts*, whereas in formerly described cases it occurred posteriorly thereto. The former more clearly indicates the real relationship.

Čelakovský (87, 94, 96, 103, 114) has repeatedly published his views on this subject from 1879 onwards, his latest paper having appeared in 1897. This investigator's treatment of the subject is perhaps broader and more comprehensive than that of any other botanist. He seems to leave no point of view, unutilized from which a possibility of penetrating more deeply into the mysteries of this complicated problem may be found.

He is a champion of the 'axillary bud' theory, first propounded by Alex. Braun, and, taking up the abnormalities described by Stenzel and Willkomm, he thoroughly overhauls and explains them, endeavouring to show that the *continuous and gradual transitions* existing between the undivided seminiferous scale of the normal condition (Fig. 1) and the first transverse pair of foliar organs of the axillary bud, along with, in many cases, the *anterior* bud-leaf (Fig. 3), as well as the agreement, in relative position of the various appendages, between this abnormal axillary bud and an ordinary axillary vegetative bud, both go to prove this theory to be the true one.

According to Čelakovský the fact that the ventral surface of the seminiferous scale is directed towards the corresponding surface of the bract is due to the law of 'Spreitenverkehrung,' by which ligules or emergences always present their ventral surfaces to that of the leaf on which they are borne, although one would have thought that a more direct and obvious reason lay in the fact that the ovules being borne on the dorsal surface of the scale, that surface must necessarily lie uppermost in order to allow both of fertilization and the escape of the ripe seed.

He strongly criticizes the theory of the abnormalities proposed by Strasburger, who regards the transformation of the disk or flattened axis (for such he considers the seminiferous scale to be) into the two first appendages and the anterior leaf of the axillary bud as the result of the struggle carried on between two opposing forces, viz. that of the vegetative development of a bud and that of the normal formation of reproductive organs. This position Čelakovský shows to be perfectly untenable; for it is impossible for an organ belonging to one morphological category, such as an axis or disk, to become transformed into those belonging to another category, such as the foliar appendages of an axis.

Eichler's theory meets with no better fate at his hands. This author's explanation of the abnormalities was this: that the pressure exercised by the axillary bud arising between the seminiferous scale and the axis of the cone, was the agency responsible for the splitting of the scale into two parts and the wide separation of these latter into the positions which they occupy, one on either side of the axillary bud. But this theory, he says, will not hold good when it is found that the splitting of the scale frequently occurs when the axillary bud is suppressed or exceedingly reduced, and also when the latter arises on *the anterior side* of the seminiferous scale, a fact which is fatal to Eichler's placental or emergence-theory, which latter is, however, best refuted by the continuous, gradual transitions which occur in the abnormalities between the seminiferous scale and the first leaf-pair plus the anterior leaf of the axillary bud. And further, if this view of Eichler's be correct, what has become, he asks, of the first transverse leaf-pair of the bud, which should occupy the position taken up by the separated parts of the seminiferous scale?

If the view that the ovule is a *flower* be correct, he reasons, then that organ ought, occasionally, in the retrograde metamorphoses, to develop vegetatively like the seminiferous scale; which, however, it never does.

For the solution of such problems as the nature of the



seminiferous scale he regards the *development* as an unreliable factor; that this is so is shown by the circumstance that Strasburger and Eichler both rest their very different views on developmental evidence. The development of the seminiferous scale, he says, is a 'heterodox' one. Nature here takes a short cut; instead of forming a bud with two distinct lateral leaves (Fig. 3), the seminiferous scale, as we know it to-day, is developed *directly*, in its highly modified form, as an outgrowth on the bract (Figs. 1 and 4). The '*systematic-morphological comparison*' he regards as a very useful factor, but the point from which it starts must be clear and certain. Eichler started out from the Araucarineae, and Strasburger from the Taxineae, hence both from quite different points. It is also important not to compare together wrong things. *Anatomical* evidence, taken by itself, is also utterly misleading, as shown by Van Tieghem's conclusion that the seminiferous scale represents the *first leaf* of an axillary shoot, an idea clearly erroneous when we know that in all Coniferae the axillary shoot always bears a *pair* of *opposite* first leaves and never a single leaf. Čelakovský's position on this point is, therefore, this, that 'the most credible and surest method of understanding questionable metamorphosed structures, which are apt, owing to heterodox development, to be misunderstood, is the *metamorphogenesis* resting on abnormalities, the neglect and wrong estimation of which is the [third] cause of the aberrant state of morphology.'

With the usual insight which he applies to the investigation of all intricate phenomena of plant-life, the author further elucidates for us the true ulterior nature of the parts of the seminiferous scale, as also of the sporangial envelopes in the various groups of the order. And so well does he account for and explain the various structures throughout this large and diversified order that, under such skilful unravelling of the knot, this diversity becomes a unity, presenting an orderly sequence of connected forms such as had never been exhibited to us in any former treatise on the subject.

Starting out with the theory, of which he was the first



exponent, that all axial appendages primarily originated by sterilization and subdivision of the sporogonium of the lower Cryptogams, and possessed, therefore, originally a *radial*, in contradistinction to a bilateral, structure, Čelakovský believes this radial structure of the sporophyll to have been characteristic of the ancestors of the Gymnosperms or, as he calls them, the Archigymnosperms. He maintains that this radial structure has persisted to the present day, in the male sporophylls of the Gnetaceae, where the sporangia are terminal; in the female sporophylls of the Cycads, where they are marginal (this being also occasionally the case on the male side, as in *Zamia*); in the sporophylls of *Ginkgo*, as well seen in the abnormal female flower described by Fujii<sup>1</sup>; in *Osmund*a, *Hymenophyllum*, and the Psilotaceae; and in the stamens of all Angiosperms. From the terminal, the sporangia came to assume a marginal position on the sporophyll until, when the latter in many plants became bilateral in structure, the sporangia were frequently relegated either to the dorsal or the ventral side, as in most Ferns, and the carpels of some Angiosperms.

In an early paper in 'Flora' he has shown that the integuments of an ovule have their origin in the *segments of the sporophyll*, the inner integument springing from *two* distinct segments, this latter fact thus easily accounting for the appearance of the integument of the Abietineae in its early stage as two distinct rudiments, misleading Baillon to assert so positively that this fact proved the ovular envelope in this group to be of the nature of an ovary and not of an integument.

Čelakovský urges that, throughout the entire order of Coniferae, no true carpel or female sporophyll is present, but that organ is reduced to a *single ovule* just as is also the case in the female organs of the Gnetaceae.

In the Taxaceae there are always two integuments of the normal form, enclosing the ovule. In the case of *Taxus*, and

<sup>1</sup> 'On the Different Views hitherto proposed regarding the Morphology of the Flowers of *Ginkgo biloba*,' 1897.

in that of *Dacrydium* and *Microcachrys* among the Podocarpeae, where the ovular integuments are distinct from each other, the ovule is spoken of as 'dichlamydeous.' In *Cephalotaxus*, *Podocarpus*, as also in Cycads, where the two integuments (consisting of the inner bony and the outer fleshy one) are intimately united, the condition is 'holochlamydeous.'

In the Araucariaceae, however (including the Abietineae, Araucarieae, and Cupressineae) the outer integument of the sporangium has assumed a *permanently vegetative* development, and is exactly comparable in this respect to the vegetatively-developed outer integument observed and described by this author in *Hesperis* and other plants. This outer integument, then, is the *seminiferous scale* or a half of it, as unlike an ordinary outer integument as it well could be, but shown to be such by the inversed orientation of its bundles, characteristic of all outer integuments (a fact first discovered by Strasburger in *Cephalotaxus*), by the fact that without it the sporangium would possess but a single integument, and thus offer an incongruous dissimilarity from the sporangium in the Taxaceae, and, not least, by the period of its development, which is always (except in *Pinus resinosa*, Soland) *subsequent* to that of the sporangium, whereas if it were a sporophyll it should be developed, naturally, before the sporangium. This dissimilarity from what obtains in the Taxaceae would again be most apparent if the seminiferous scale or its half were regarded as a sporophyll or carpel, for no such organ is found in the above-named section of the order.

As regards the homology of the sporangial envelopes with structures found amongst Vascular Cryptogams, the inner integument corresponds to the indusium of Ferns, *Isoëtes*, &c., while the outer integument is the homologue of the ligule in *Isoëtes*, *Selaginella*, &c., a view which is so far identical with that of Sachs and Eichler, who regarded the seminiferous scale as of ligular nature.

While in the Abietineae the seminiferous scale very frequently consists of the sporangial representatives of the first pair of leaves of the axillary bud fused with that of the *anterior*

leaf of the latter (this anterior leaf being twisted round through an angle of  $180^\circ$ ), in the Araucarieae (Fig. 2), where the seminiferous scale, bearing a single ovule, is obviously not a compound but a single organ, the *scale consists solely of the anterior leaf* or its sporangial representative, the first leaf-pair of the axillary bud being entirely absent. This is the explanation of the occurrence of a single sporangium in Araucarieae. The same explanation is, in all probability, also applicable to the case of the Podocarpeae, where but a single sporangium also occurs. In the Taxodineae the seminiferous scale may frequently consist of several parts, representing the fusion of *more* than three leaves, or their sporangial representatives, of the axillary bud. Abnormalities, either in this group or in the Cupressineae, are almost unknown. But, in support of the view as to the origin of the seminiferous scale in the Taxodineae, Engelmann, in America, found a proliferated cone of *Sequoia* which clearly exhibited the compound nature of the scale, while Alex. Braun, in *Taxodium*, *Cryptomeria*, and *Glyptostrobus*, found an axillary bud replacing the scale. Although no abnormalities have ever been found to prove it, there can, nevertheless, be little doubt that in the Cupressineae, the seminiferous scale, which is almost completely fused with the bract, has the same origin as it has in the Abietineae. But a difficulty would seem to arise, owing to the presence in some species of *Cupressus* of *great numbers* of sporangia belonging to a *single* seminiferous scale, if the latter is to be interpreted as representing the fused, vegetatively-developed outer integuments of two sporangia. But the difficulty vanishes when confronted with the fact that in *Hesperis* was found a vegetatively-developed outer integument bearing *several inner integuments* or ovules, one on each vein (Fig. 7), and each corresponding to a lateral segment of the leaflet.

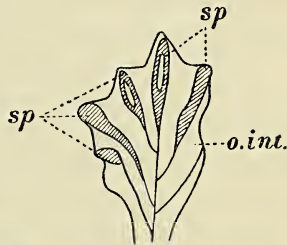


Fig. 7. Vegetatively-developed outer integument (*o.int.*) of *Hesperis*, showing several inner integuments (*sp*) borne on the lateral veins (after Čelakovský).



The female sporophylls of Coniferae belong to branches of a higher grade than those bearing the male sporophylls. The male sporophyll corresponds to the *bract* subtending the axillary shoot (now represented solely by the seminiferous scale), which latter bears the female sporophylls, i.e. their sporangial representatives. That this represents the real relationship is shown by those abnormalities in which the 'bracts' bear pollen-sacs on their lower surface, when the seminiferous scale, or female part of the flower, tends to disappear, and this generally in the lower part of the cone, whereas in the upper part the female parts develop in the normal way and the bracts bear no pollen-sacs. The mon-axial cones were originally hermaphrodite, like the strobili of *Selaginella*, or the flowers of most Angiosperms<sup>1</sup>.

The real relationships, as they are regarded by the author, may be stated thus:—the *female inflorescence* of the Coniferae is equivalent to the *axillary brachyblast* of *Ginkgo*, and the main axis of Cycads. In the Taxeae the inflorescence consists of a primary axis bearing a secondary axis (the 'flower') in an axillary position; this 'flower' is unique among Conifers from the fact that, like the Cycads, it bears a number of sterile bracts in its lower portion; the sporangium has become shifted from its ordinary lateral position, into one purely *terminal* to the axis, the sporangium itself being the sole representative of the sporophyll which was the *uppermost* appendage of the axis. This 'flower' is exactly equivalent to that of *Ginkgo*.

<sup>1</sup> Von Mohl observed a highly interesting case in an androgynous cone of *Picea alba*, Link, which bore reduced and sterile seminiferous scales in the axils of bracts, one of which latter bore two pollen-sacs on its lower surface, while on its upper surface appeared two wing-shaped projections, one on each side at the base, but nearer the upper than the lower side, which, in mode of adherence and direction, greatly resembled sporangia, and are in fact the sporangial leaf-segments of the sporophyll. So that this would, therefore, be a hermaphrodite scale, and possibly represents what would have been the original structure of the female inflorescence when the 'bracts' bore the sporangia, and *no axillary shoots were developed*, these latter appearing later, and themselves subsequently becoming modified into the present structure. These sporangia, from their position on a sporophyll, and having no seminiferous scale attached to them, must necessarily be holochlamydeous, like the sporangium of *Cephalotaxus*, and not monochlamydeous like that of the Abietineae.



It is highly interesting, as indicating a reversion to the ancestral condition exhibited in modern Cycads, that *Torreya* has been known to produce, besides the lateral secondary axes, a 'flower' terminal to the primary axis. This latter fact, and the presence of the sterile bracts in the 'flowers,' clearly shows the Taxeae to be one of the very oldest groups of the order. In *Cephalotaxus* 'the normal flower consists of two lateral . . . carpels formed of ovules pure and simple, accompanied usually by a median third, but sterile, carpel. In the Podocarpeae the flower has been reduced to a single axial appendage, i.e. to an ovular carpel, and at the same time to an ovule actually axillary, and often carried up upon a bract.' In the Araucarieae the structure is the same (Fig. 2). In the Abietineae the seminiferous scale is 'a symphyllodial structure, consisting of three fused appendages [two in *Picea*] of an axis, of which the two lateral are fertile carpels [reduced to sporangia] fused together to form the 'crista' of the seminiferous scale, while the third median leaf—the median knob of the first rudiment—remains sterile, and either aborts or, fused with the two other fertile carpels, forms the keel and mucro (in *Pinus*).' In the Taxodineae and Cupressineae the structure is essentially the same.

The modifications which have in later times supervened amongst the Coniferae have been, firstly, the relegation of the flowers from the terminal, as in Cycads, to the axillary position, as in *Ginkgo*; secondly, the supplanting of the hermaphrodite by the diclinous condition, while finally, in some groups, the female came to belong to a different rank of branching from that of the male 'flowers'; and, thirdly, the adoption by the sporophyll, in many cases, of a bilateral instead of a radial symmetry.

The structure which prevails throughout the Coniferae is found to be uniform with that prevailing in the other two groups of Gymnosperms. In the Cycads the sporophylls, with the exception of those of the female plant of *Cycas*, still retain in part their radial symmetry, while the sporangia possess a holochlamydeous or double integument. In the Gnetaceae

the male sporophylls have preserved their radial symmetry more or less completely; whilst the female are, as in Coniferae, reduced to single ovules, each with two integuments.

#### GENERAL SUMMARY.

In conclusion I give in brief the most important of the numerous theories relating to the nature of the 'seminiferous scale' and the 'ovule' respectively, which have been cited at greater length in the foregoing pages.

#### *Views on the Nature of the Seminiferous Scale.*

LINNAEUS (1737, 1792): The seminiferous scale of the Abietineae is a *calyx*. In *Juniperus* the ventral tip of the scale is the *corolla*.

JUSSIEU (1789): The bract is a style; the seminiferous scale a *bilocular ovary*.

MIRBEL (1810-15): The seminiferous scale is a *peduncle*.

BROWN, R. (1814-66): The seminiferous scale is an *open carpel*.

SCHLEIDEN (1839-43), BAILLON (1860-65), STRASBURGER (1872-79), MASTERS (1891): The seminiferous scale of Abietineae is an *axis*, either in the form of a *placenta* (Schleiden), an *axillary bud* (Baillon), a *disk* or (in the Cupressineae and Podocarpeae) an *axillary bud* (Strasburger), or a *half-cladode* (Masters).

SACHS-EICHLER (1868, 1881-82): The seminiferous scale is a *ventral outgrowth* from the open carpel, of the nature of a *placenta* or *ligule*. In the Cupressineae, Taxodineae, and Podocarpeae no differentiated ventral outgrowth exists; but the scale as a whole is an *open carpel*.

VAN TIEGHEM (1869): The seminiferous scale is the *first and only leaf of an axillary bud*. In the Podocarpeae this leaf is represented solely by the ovule. In the Taxeae the same thing occurs on secondary shoots.

BRAUN (1853-75), CASPARY (1860), PARLATORE (1864), ØRSTED (1864), VON MOHL (1871), STENZEL (1876), WILKOMM (1879), ČELAKOVSKÝ (1879-97): The seminiferous scale consists of the *two first leaves (or their ovular representatives) of an axillary bud*. This view is founded chiefly on the *abnormalities*.

DELPINO-PENZIG (1894): The seminiferous scale consists of *the two lateral lobes of the bract bent inwards and fused by their margins*.

#### *Views on the Nature of the Sporangium.*

LINNAEUS, JUSSIEU: Pistil.

MIRBEL, RICHARD: Complete flower, consisting of *calyx* or *cupule* (our integument), and *ovary* (our nucellus), i. e. a caryopsis or nut, whose pericarp is fused with the integument of the seed.

BROWN, R.: Ovule. First exponent of the 'Gymnospermous' theory.

BAILLON: Ovary. View founded on the *development*.

PARLATORE: Ovary.

VAN TIEGHEM: Ovule.

SPERK: Ovary.

BRAUN, A.: Ovule.

STRASBURGER: Ovary (earlier view); ovule (later view).

EICHLER: Ovule.

ČELAKOVSKÝ: Ovule (representing the entire sporophyll).

It will thus be seen how various are the theories which have from time to time been put forward on this subject. It will be said by many that the problems connected with it remain still unsolved, but it seems to me that their solution depends very largely on the individual insight of each one, as to how he interprets facts placed before him. Personally, I incline strongly to the views put forth by Čelakovský, the latest author who has written upon the subject. They are views which, in their interpretation of organs, appear the least to clash with our modern morphological conceptions, and which, from their breadth of view and comprehensiveness, appeal forcibly to the scientific sense. It seems to me that the evidence accruing from the abnormalities, in which *a most gradual transition* exists between the normal seminiferous scale and the two or three first leaves of an axillary bud, is ample for the solution of the problem as to the position of the seminiferous scale between the bract and the axis (Fig. 1). I hold both with Velenovský and Čelakovský that this evidence is eminently to be relied upon, because 'in all stages of the scale-metamorphosis we find a definite law and the greatest regularity of development,' and 'such a regularity can never be an equivocal, pathological, and casual phenomenon.' This will hardly apply to the evidence derived from the anatomy or the development, which, as Čelakovský has shown, is quite untrustworthy. So that, speaking for myself, this part of the whole problem is already solved.

With regard to the vexed question of Gymnospermy which is intimately bound up with and, indeed, inseparable from that as to the nature of the seminiferous scale, the view propounded by Čelakovský is once more distinguished by its comprehensiveness of outlook, and by the fact of its suggesting a unity of morphological structure throughout,



not only the order Coniferae, but all three orders of the Gymnosperms, where before we but recognized a considerable amount of diversity. Hitherto, it has been regarded as a matter beyond all controversy that the sporangium in Coniferae possesses but a single integument; that the fleshy envelope of the Taxeae is either of the nature of a disk or *special* envelope or aril of no morphological value, as in *Taxus*, or that it represents the outer layer or layers of the single integument, as in *Cephalotaxus*, comparable to the case of the Cycads. Yet the sporangium of *Podocarpus* was, strangely enough, regarded as unique in possessing two integuments. Čelakovský's theory, however, introduces us to a plan of perfect uniformity of structure in this respect throughout the entire order. For he maintains the constant presence throughout the order of not one, but two integuments to the sporangium, thus abolishing the awkward inconsistency of the presence of such extra and unusual organs as an aril, and the unique possession by *Podocarpus* of two integuments.

There is nothing unreasonable in supposing outer integuments capable of becoming modified in the direction of vegetative development to subserve the special adaptive function of a protective covering to the sporangia. The outer integument, forming an integral part of the sporangium, would, probably, if developed as Čelakovský suggests, as a protective scale, afford greater security to that organ than would the bract, in whose axil the sporangium would lie more or less independently of the latter or, at least, not so intimately bound up with it as it is with the seminiferous scale.

In view of the comparatively one-sided nature and narrow scope of the views advanced by other writers on this subject, it appears to me probable that, in the near future, a theory of the structure of the female 'flower' in Coniferae, if not absolutely identical with, yet approximating most nearly to that which in recent years has been elaborated by Čelakovský, will occupy a paramount place in the minds of the foremost botanists of the day.



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# The Structure and Development of *Triglochin maritimum*, L.

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With Plates VI and VII.  
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*TRIGLOCHIN* has been somewhat neglected by botanists up to the present time, very little work indeed having been done upon it. The material upon which this investigation was carried out was collected at Yarmouth, in the Isle of Wight, by Prof. Farmer, and at Tilbury, in Essex, where it grows in abundance on the banks of the Thames.

The genus contains about twelve species, many of which are Australian, and is distributed throughout the Temperate regions of the Earth.

*Triglochin maritimum*, L., grows in salt marshes along the coast and estuaries of temperate climates. It possesses a rhizome of a fair thickness, bearing at its apex a dense tuft of leaves, which are generally half-cylindrical in shape, and may attain quite a considerable length. The inflorescence is a raceme; the flowers are very numerous, and borne upon very short pedicels.

The fruit consists of six carpels, which separate one from

[*Annals of Botany*, Vol. XIV. No. LIII. March, 1900.]

another when maturity is reached. The fruits of this plant are much stouter and shorter than are those of *Triglochin palustre*, L.

#### ANATOMY.

The existing accounts of the anatomy of *Triglochin maritimum* are very scanty. The only descriptions which have been found relating to the anatomy, taken as a whole, are embodied in an account by Chatin (3), which was published in 1862, and in a paper by Van Tieghem, published in 1870 (14 a), the latter being mainly concerned with the structure of the root. These two papers are the principal sources of our information of the structure of the plant, and they leave much to be desired in the matter of completeness. Chatin, in the paper just referred to, gives a very short account of the general anatomy of *Triglochin maritimum*, and adds a few remarks on that of *Triglochin palustre*. He gives very few details, and merely describes the general disposition of the tissues, briefly noting a few characters of each. In speaking of the root, he correctly states that spiral vessels are absent.

Van Tieghem (14 a), in discussing the structure of the roots, states that the xylem-strands do not meet in the centre of the root, but are separated by parenchymatous cells; the pericycle is described as a continuous layer, with the sieve-tube of each phloem-group abutting on it, and the companion-cell does not appear to have been noticed. These statements have not been verified in this investigation. Van Tieghem also calls attention to the fact that the lacunae in the inner cortex show signs of lysigenous development.

#### THE RHIZOME.

This organ may attain a considerable length; how long, it is impossible to say exactly, for the rhizomes are very brittle, and the plant grows in a very heavy soil, so that it is extremely difficult to clear away the earth without breaking them; pieces of rhizome, however, have been found as long as 7 cm. The root-stock is not very thick; the older portions have a diameter

of 4 mm., while in the more apical regions a diameter of 8 mm. is quite common in well-grown plants ; the reason of this lies in the fact that here the cells are younger, and consequently more turgid.

The younger parts of the rhizome are covered, especially around and just below the apex, with the persistent bases of old leaves, which are quite chaffy in character. The rhizome frequently forks into two branches, each end bearing a crown of leaves and possibly flowers.

A transverse section of an old rhizome shows a structure such as is diagrammatically figured in Pl. VI, Fig. 1. From this figure it may be seen that the underground stem is bounded by a mass of brown cells, to the inside of which succeeds a layer of sclerotic elements, which are lignified and extremely hard. These cells are much pitted with simple branched pits. It will also be observed that these strongly lignified cells occur in various other places, the more central regions of the axis of the older parts of the rhizome being always occupied by cells of this character.

The cortex consists of rounded cells loosely arranged in a somewhat radial manner, with numerous intercellular air-spaces (Fig. 2). The cells of the cortex of plants collected in the winter and spring are densely packed with starch-granules, and so also are the parenchymatous cells of the central cylinder.

The endodermis is represented in Figs. 2 and 3, Pl. VI : in the older parts of the rhizome it is extremely well marked, forming a continuous ring of cells much thickened on their radial and inner walls ; this layer, in fact, is remarkably similar to the endodermis such as is met with in the roots of *Dracaena* or *Iris*.

The endodermis in the younger parts of the rhizome is not easy to make out in the ordinary stained microscopic preparations, but its presence can easily be demonstrated by means of the usual test of iodine and sulphuric acid.

The central cylinder has a large number of vascular bundles, which, when fully developed, resemble those of the concentric



type (excepting that here the xylem is interrupted by parenchymatous passage-cells, see Fig. 2), the xylem surrounding the phloem, as 'occurs in the lower ends of the leaf-trace bundles of many, but not of all, rhizomes of Monocotyledons, where they lie at the periphery of the bundle-cylinder in the stem, e. g. *Iris germanica*, *Cyperus aureus*, *Papyrus*, *Carex arenaria* (but not, for example, *C. disticha* and *C. hirta*), *Acorus calamus*, and *A. gramineus*<sup>1</sup>.

A transverse section of a fully formed bundle is represented in Fig. 2, Pl. VI. The xylem consists of tracheides, which are, comparatively speaking, of great length and frequently branched; sliding growth has gone on to a great extent, so that these elements are much interwoven; hence it is extremely difficult to isolate them.

The protoxylem consists of a few elements thickened in the usual manner; it is generally placed towards the centre of the axis, although cases have been observed in which the protoxylem occupies a more lateral position.

The ring of xylem in such a concentric bundle is not, as already stated, complete, but is separated into masses by parenchymatous passage-cells; five such passage-cells are frequently present.

The phloem is exceedingly well marked and regular, the sieve-tubes (*s. t.*) and companion-cells (*comp. c.*) being very distinct; the former are apparently void of contents, and the latter filled with protoplasm, with a large and well-defined nucleus.

The parenchyma surrounding a bundle may sometimes be thickened in various places; such a case is shown in Fig. 2 (*th. par.*).

The ground-tissue in which the bundles are embedded is made up of parenchymatous cells, generally rounded in shape, but often elongated between two adjacent vascular bundles.

As the rhizome increases in age the central parenchymatous cell-walls of the vascular cylinder thicken up and become

<sup>1</sup> De Bary, *Comp. Anat. of the Phanerogams and Ferns*, Eng. ed., 1884, p. 339.



lignified to such an extent that the lumina become almost obliterated ; in this manner the extremely hard sclerotic cells already referred to become differentiated.

The course of the leaf-trace bundles in the rhizome calls for no special comment, inasmuch as it somewhat resembles the type exemplified by the Palms, that is to say, the bundles, after coming in from the leaves, travel obliquely through the cortex to the central cylinder, where they curve towards the centre and then pass out to the periphery. In *Triglochin* the course of these bundles is very complicated, inasmuch as numerous branchings and anastomoses take place.

A transverse section of the rhizome shows numerous small bundles arranged in the peripheral part of the central cylinder. These are connected by horizontal commissures to form a *réseau radicifère* such as obtains in many Monocotyledons. These peripheral bundles run roughly in a longitudinal direction ; but in the region of insertion of a root they are united transversely, so that a root may be connected with at least one-half of the total number of bundles, of which there may be as many as twenty-five to thirty.

One feature of interest connected with the anatomy of the plant under consideration lies in the occurrence of occasional cell-divisions recalling those of a cambium. These cambia were not at all common ; the best example seen is represented in Fig. 3, Pl. VI. From this figure it will be seen that the cambium is well marked and has been formed from the pericycle ; it is situated opposite a young bundle on its outer side. The other examples seen were cambial divisions in single pericyclic cells ; they were not sufficiently developed, however, to throw much light upon the subject.

The part of the rhizome from which the section containing this cambium (Fig. 3) was cut was not by any means young, as is seen by the greatly thickened endodermis ; unfortunately it cannot be said exactly how old this part of the rhizome was, inasmuch as the portion was an isolated piece of stem. There were no indications of the cambium being due to pathological effects, the cells around being, as far as could be judged from

their appearance, quite healthy and normal. It may perhaps be argued that in *Triglochin maritimum* we have a case of incipient secondary thickening which is found developed to a much greater extent in Monocotyledons like *Aristea*, *Dracaena*, *Yucca*, &c., and in this respect it is interesting to recall remarks by Dr. Scott and Mr. Brebner<sup>1</sup>. Writing of *Aristea corymbosa*, Benth., they state their opinion that the secondary thickening 'has originated *de novo* probably at a comparatively recent period.' And again, 'it is very probable that the first origin of secondary growth may be taking place in some of the Monocotyledons at the present day.'

Petersen<sup>2</sup> expresses much the same opinion in his paper on secondary thickening in Monocotyledons; he says: 'En examinant un nombre assez grand de tiges de plantes monocotylédones je me suis pourtant convaincu de ce qu'il se trouve une transition plus graduelle de ces tiges, où l'on n'a point du tout pu démontrer une formation méristématique, jusqu'aux phénomènes que nous connaissons chez le *Dracaena*.'

*Triglochin palustre*, the rhizome of which is much smaller than that of *T. maritimum*, has been examined for cambium, but so far without success. It is quite possible, however, that some of the other species of *Triglochin*, especially those with a well-developed rhizome, may be found to show a more advanced secondary thickening.

Unfortunately it was not possible to obtain fresh material of other species of *Triglochin* for this investigation, although a small quantity of herbarium material was obtained of the following species: *T. triandrum*, Mich., *T. procerum*, R. Br., *T. Maundii*, F. Muell., and *T. montevidense*, Spreng.; but this material was very unsatisfactory to work with, and it was not possible to obtain reliable evidence either way.

<sup>1</sup> Scott, D. H., and Brebner, G., on the Secondary Tissues in certain Monocotyledons. *Annals of Botany*, Vol. vii, 1893.

<sup>2</sup> Petersen, O. G., Remarques sur la croissance en épaisseur et sur les régions anatomiques de la tige monocotylédone; French résumé. *Botanisk Tidsskrift*, Vol. xviii, 1892, p. 125.

## THE FLOWERING STEM.

Fig. 4, Pl. VI, illustrates in a diagrammatic manner a transverse section of a flowering stem.

The epidermis is well marked, consisting in young specimens of thin-walled oval cells, as seen in transverse section; in the older specimens a cuticle is developed, which is longitudinally ridged in much the same way as in the leaf to be described later. Stomata occur in the epidermis, but they are not very numerous; the guard-cells are sunk a little below the general surface of the stalk, and are very similar to those of the leaf. The cortical cells immediately within the epidermis contain chlorophyll-corpuscles.

In the cortex of the lower regions of the stalk a large number of air-spaces occur, recalling the arrangement met with in the spongy mesophyll of an ordinary leaf. These air-spaces are more regular in the younger regions of the stem, where their appearance is very similar to that of the air-spaces in the stem and petiole of purely aquatic plants.

The structure of the vascular bundles, which are here of the collateral type, is illustrated by Figs. 5 and 6, Pl. VI. The phloem, as is usual with Monocotyledons, is very regular and well marked. In some bundles, more especially in the older ones, a few elements in the phloem-parenchyma become lignified; such a case is shown in Fig. 6. In Fig. 5, illustrating the structure of a younger bundle, it may be seen that primary meristem, between the phloem and xylem, persists for some time before passing over into permanent tissue: this is of some interest, inasmuch as the same persistence of meristematic tissue is found in many plants belonging to the Ranunculaceae. This cambial arrangement is not so well marked in older bundles, for, of course, the elements have been transformed into permanent tissue.

The xylem calls for no special comment.

As the flower-stalk grows older the cells of the ground-tissue surrounding the separate bundles form a lignified sheath,



so that in quite old stems there is a ring of strongly lignified tissue, the cells of which often exhibit well-marked simple pits.

The medulla of the young flowering stems has a large number of air-spaces developed in it; these air-spaces are arranged in a very regular manner, and their appearance recalls that which is generally associated with aquatic plants. The older scapes are frequently hollow, this being due to the inequality of growth of the outer and the more central regions of the stem.

#### THE LEAF.

The leaves of *Triglochin* are acicular in shape, with a sheathing base, and are arranged in an equitant manner. In transverse section they are generally semicircular in outline. They may attain a length of 15–16 cm.; the breadth of course varies in the different regions of the leaf. The greater diameter of a half-cylindrical leaf was 3 mm., and the lesser  $1\frac{1}{2}$  mm., measured across the middle region.

The structure of the leaves is illustrated by Figs. 7 and 8. Fig. 7 shows the general structure in a diagrammatic manner. Surrounding the two younger leaves the sheathing base of an older one is seen; from this figure it is also apparent that air-spaces, which are schizogenous in origin, are largely developed, especially in the more central regions. Embedded in the ground-tissue are numerous collateral vascular bundles, the larger ones lying nearer to the median plane of the leaf (see Fig. 45, Pl. VII). These bundles are very similar in structure to those already described for the peduncle, and hardly call for a detailed account here.

On comparing the positions of the separate bundles, in the sheathing and upper regions respectively, of a leaf, it will be seen that as the bundles pass from the base towards the apex they alter very much as regards their relative positions; hence it was desirable to trace out their course in some detail.

Several bundles pass up from the rhizome to each leaf, e. g. all those represented in diagram *A*, Fig. 45, Pl. VII, were found to run into the stem as separate leaf-traces.



Fig. 45 illustrates the positions of the bundles in the upper and lower regions of the leaf.

A transverse section of a leaf, just above its junction with the stem, shows the general arrangement of the bundles, the largest one (1) being in the centre. Intercalated between the larger bundles are smaller ones, sometimes consisting of quite a few elements; these small bundles may completely disappear higher up in the leaf. The diagram *A* in Fig. 45, Pl. VII, shows the bundles in the upper regions of the sheathing leaf-base; all the bundles here represented are to be found in the higher portions of the leaf, although their relative positions are different. In order that this may be followed more easily the bundles have been numbered, those having the same number in the two diagrams being identical.

As the bundles pass up the leaf the first changes in position noticed are: 2 and 11 in the sheathing base move towards the convex side, and the bundles of the horns of the crescent gradually travel obliquely up towards bundle 1.

The large bundle 1 remains in practically the same central position.

The bundles 3 and 10 travel in such a way as to retain their relative position with regard to bundle 1. Passing upwards from the sheathing base, the bundle 9 moves towards the plane (ventral) surface of the leaf, taking up a position below and a little to the right of 1; bundle 4 does likewise, taking up a similar position on the left side of 1. Before the bundles 4 and 9 have taken up their final position (shown in *B*), it was found that three new bundles arose near the periphery of the convex (dorsal) side of the leaf, and almost opposite to the bundles 1, 3, and 10; they are indicated by the letters *a*, *b*, and *c* in diagram *B*.

All the bundles have now left the sheath; a branch is then given off from 6 and joins up with 4, and subsequently a branch from 7 unites with 9. Bundle 6 now gives off another branch (6'), which runs up the leaf in a position between the two first-named bundles. Bundle *e*, which occupies a similar position to 6' in the opposite side of the leaf, was found not to be

a branch from 7, but to arise independently<sup>1</sup>. It seems very probable that this may be a special anomalous case, as in other respects the behaviour of the bundles is similar on the two sides of the leaf; so that one would expect a branch from 7 to occupy the position filled in this particular leaf by the bundle *e*.

There is a certain amount of variation as to the course of the bundles in different leaves; for example, it was found that in another leaf the bundle corresponding to 1 gave off a branch, which finally occupied a position similar to that of 9 in the leaf described above. Then, again, the adjacent bundles of some leaves, especially the larger ones, anastomose somewhat freely in the transitional regions between the sheath and the blade. The main points, however, expressed above are typical.

The structure of the assimilatory region of the leaf is illustrated in Fig. 8. The epidermis is well marked and, in the older parts of the leaf, is covered with a cuticle of fair thickness. This cuticle, like that of the flowering stem, is longitudinally ridged, there being from three to five ridges to each epidermal cell. The stomata are numerous, showing a typical structure, and are placed slightly below the general level of the epidermis. The palisade-parenchyma is several layers in thickness, and is made up of oval-shaped cells with small air-spaces between them.

Glandular bodies are developed in the axils of the leaves; in shape they are triangular and flattened, and very frequently quite numerous, as many as seventeen having been counted in one leaf-axil: they are sessile and made up of parenchymatous cells, with dense contents and well-marked nuclei, somewhat larger than are found in the non-glandular vegetative parts of the plant.

Irmisch (10) was the first to call attention to these glands in the leaf-axils of *Triglochin maritimum*.

<sup>1</sup> No definite connexions were to be made out between these and the adjacent bundles; this may be due to the fact that the leaf was quite a young one, and still retained its merismatic condition. It might also be noted that these bundles, *a*, *b*, and *c*, are not found in all leaves.

In the paper referred to he describes their form and arrangement. Similar structures occur in other natural orders, e. g. Hydrocharidaceae and Callitrichaceae (see Caspary, 2, and Hegelmaier, 9.)

At the base of each glandular body there is seen, in longitudinal section (Figs. 10 and 11), a layer of cells, thickened in much the same manner as an endodermis. This layer of endodermoid cells is continuous across the plane of attachment of the gland, and is not found elsewhere.

The thickenings are somewhat difficult to make out in the very young glands, but with advancing age the thickenings increase considerably until they are as represented in Fig. 11; so that when the gland is no longer necessary to the plant and drops off, the part of the cortex with which it was in contact is fully protected. This very interesting fact does not appear to have been noticed by earlier observers.

In connexion with the cutting off of these glands by the development of a special tissue, it is interesting to note that Miss E. Dale (5) has found that a somewhat analogous process obtains in the intumescences situated upon the stem of *Hibiscus vitifolius*, the outgrowths being there cut off by cork, which arises in the lowest row of daughter-cells derived from the original epidermis.

The secretion of these glands appears to be mucilaginous. They are stained pink with corallin-soda, and blue with aniline blue, although these reactions were not so well marked as those obtained with the glands of *Rumex*, which were tested at the same time and with the same reagents. Oils and tannin appear to be absent, for no reactions took place when they were treated with osmic acid, chromic acid, potassium bichromate, and ferrous sulphate and nitric acid.

#### THE ROOT.

The roots of *Triglochin* are adventitious, and arise acropetally from the rhizome; they are very numerous, and may attain a length of 10 to 11 cm., with a diameter of about 1 mm.



The structure of these organs is illustrated by Figs. 12, 13, and 14, Pl. VII.

The piliferous layer is well marked, the cells being generally oblong in shape, whilst those which have grown out into root-hairs are nearly square. This layer may become lignified in old roots. The root-hairs, on becoming functionless, do not necessarily die off, but may become thickened and persist. According to Van Tieghem (14*b*), the root-hairs are sometimes formed very near the end of the root, just behind the calyptra.

Immediately beneath the piliferous layer is a well-marked exodermis (Fig. 12 *exo*), normally one cell in thickness, but here and there forming a double layer.

Beneath the exodermis are two or three layers of rounded thickened cells, with no intercellular spaces; these, however, occur between the innermost row and the subjacent cortical layers.

The cells of the inner cortex are arranged in a very regular radial manner, especially in the younger regions of the roots. They are rounded in shape, with lozenge-shaped schizogenous air-spaces between them; these cells and their accompanying air-spaces gradually grow smaller in passing from the periphery to the central cylinder.

The endodermis is extremely well marked, having much the same appearance as the endodermis of the rhizome, the radial and inner walls being very strongly thickened (Fig. 13).

In the older regions of the roots large lacunae are found in the outer parts of the inner cortex; these air-containing spaces show some signs of lysigenous development. Van Tieghem draws attention to this.

The vascular cylinder may be pentarch, hexarch, or heptarch. Van Tieghem (14*a*) states that the radiating vascular strands do not meet in the centre, being separated by parenchymatous cells; the material examined did not bear out this statement, for it was found that in every case the xylem-strands did meet in the centre.



In common with the roots of many other Monocotyledons, e.g. *Aponogeton* and *Potamogeton lucens*, according to Van Tieghem (14*a*), each phloem-group in the roots of *Triglochin maritimum* is reduced to one sieve-tube with its companion-cell.

In the very young regions of the roots the mother-cell of the sieve-tube and companion-cell is seen as a six-sided element, somewhat larger than its neighbours. As it increases in age a periclinal wall arises, dividing the cell into an outer cell, which is the sieve-tube, and an inner segment, which is the companion-cell. The sieve-tube abuts directly on the endodermis, thus interrupting the pericycle at this point.

It is somewhat difficult to demonstrate the presence of the sieve-plate; not one was clearly seen in the younger parts of the root; Fig. 14 illustrates these structures as they were found in the oldest part of a root embedded in the cortex of the rhizome. From this figure it may be seen that the sieve-tube is undoubtedly next the endodermis, and in this case the sieve-plates are typical and of the simple type of structure.

Van Tieghem figures and describes the pericycle as a continuous layer, with the sieve-tube abutting on it, and he does not appear to have noticed the companion-cell. In all cases the sieve-tube is in contact with the endodermis and interrupts the pericycle, and the companion-cell is easily recognized<sup>1</sup>.

In longitudinal section the protoxylem is seen to be composed generally of annular elements.

The apex of the root of *Triglochin maritimum* so closely resembles that of *Zea Mais* that it hardly calls for an extended description.

The roots do not frequently branch, so that preparations showing the first origin of lateral roots were not obtained. From young lateral roots, still embedded in the cortex of the mother root, it appears that they arise from the pericycle opposite the protoxylem-groups.

<sup>1</sup> It would perhaps be as well to note here that Figs. 33 and 35 in Van Tieghem's paper appear to be wrongly numbered. Fig. 33 seems to refer to *Aponogeton*, and Fig. 35 to *Triglochin*, instead of vice versa.

## INFLORESCENCE AND FLOWERS.

The inflorescence of *Triglochin maritimum* consists of a raceme, the flowers being attached by very short pedicels to the peduncle. The flowers in each inflorescence are very numerous, and are especially crowded at the apex of the flowering stem.

An interesting fact, which has already been recorded in connexion with the inflorescence of this plant by Van Tieghem (14*b*) and other observers, is that the apex of the flowering stem is very frequently occupied by a flower.

The individual mature flowers, which have no bracts, are greenish in colour, although they sometimes have a very decided purple hue.

The perianth consists of six segments arranged in two alternating whorls of three lobes, the segments of the outer ring being somewhat larger than those of the inner. The stamens are six in number, arranged in two whorls, alternating with those of the perianth.

The ovary is made up of six carpels which, early in life, become attached to one another, separating when the seeds are ripe. Each carpel has a single feathery stigma, and contains one seed. The fruit of *Triglochin maritimum* is not nearly so long as the fruit of *Triglochin palustre*, but is very much broader.

## FLOWER-DEVELOPMENT.

Cordemoy (4) worked out the floral development of *Triglochin palustre*, and his account, which unfortunately is not illustrated, closely corresponds to what has been found to obtain in *Triglochin maritimum*, excepting, of course, the fact that in *Triglochin palustre* one whorl of carpels is suppressed.

The flower first arises as a spherical outgrowth, which is illustrated in Fig. 15.

The perianth is the first of the floral structures to arise, and, for the sake of clearness, the components of the outer

perianth whorl will be spoken of as the sepals, and the individual parts of the inner ring as the petals.

The individual whorls arise in acropetal succession from the first primordium.

*Calyx.* The anterior sepal arises first; it takes its origin from the basal part of the spherical primordium already referred to. The shape of this young sepal is somewhat crescentic, and of a fair thickness. Very soon after the first sepal has made its appearance the two lateral sepals arise, the left-hand one originating slightly before its fellow on the right-hand side (Figs. 15-18).

*Corolla.* After the sepals have grown slightly the petals commence to put in an appearance. They arise in positions alternate with those of the sepals, the posterior petal originating first; it does so as a small rounded outgrowth, which is more or less flattened. The shape of these young petals is not nearly so elongated as that of the young sepals; they are also very much smaller, and arise in a position situated higher up upon the original primordium. The other petals follow very quickly—indeed almost simultaneously—so that it is somewhat difficult to say which of the two lateral petals develops first: as far as can be made out, the petal which occupies a position between sepals 1 and 2 is the first to appear, and is very quickly followed by the other. It sometimes happens, however, that the first lateral petal to arise is that lying between sepals 1 and 3, so that a certain amount of variation may occur (Figs. 19 and 20).

*The Androecium.* As soon as the segments of the perianth have become differentiated, the stamens make their appearance.

The outer staminal ring, as already pointed out, is opposite the outer perianth whorl (sepals), and it arises before the inner ring of stamens as oval-shaped masses of tissue (Fig. 21). It seems very probable that the appearance of each stamen follows that of the perianth segment to which it is opposite, for it was observed that the stamen superposed on the first sepal was the first to originate. It was not seen which of the



two lateral stamens arose first, but, judging from their size, it seems extremely probable that the one opposite the second sepal arises slightly before the other, which follows it very quickly.

When the stamen has attained a certain size, increased lateral growth takes place, so that a two-lobed structure results, and in a short time the stamen becomes quite two-lobed, each lobe being slightly constricted, owing to the formation of the two loculi.

The inner staminal whorl arises after the first has been developed, the stamen opposite the posterior petal being the first to arise.

By the time the stamens are all developed, the sepals have increased very much in size, so that each one forms a hood, as it were, over the stamen opposite it (Figs. 21 and 22).

*Gynoecium.* As in the case of the stamens, the carpels also are arranged, though not quite so obviously, in two whorls. A carpel first arises as a somewhat flattened protuberance; growth gradually extends up the dome-shaped receptacle, so that a young carpel has a shape somewhat like that of a horse-shoe.

In the carpels the suture along which the coalescence of the two horns has taken place is indicated by a faint line (Fig. 25).

The walls of the carpel grow upwards, so that a hollow vessel, somewhat of the shape of a pear, results. After a time, the outer part of the wall of the carpel grows more quickly than its inner region; hence the aperture at the top becomes placed somewhat nearer the centre of the flower. In this way a hood-shaped structure is produced (Figs. 25 and 26). It is about this time that the hairy stigmatic surface arises.

The ovule originates as a dome-shaped mass of tissue, while the carpellary walls are still in a primitive state.

The ovule is basilar in origin, and anatropous in character.



## THE EMBRYO-SAC.

Hofmeister (9) has described the embryo-sac, &c., of this plant. He draws attention to the anatropous ovule, and to the fact that the remains of the nucellus surrounding the embryo-sac are of some thickness.

Writing of the embryology he mentions that the oospore frequently increases in size before division takes place. In the segment-cell which immediately abuts on the end-cell of the proembryo, and which proves to be the mother-cell of the embryo, divisions take place in three directions of space.

He also draws attention to the endosperm, which, as a closed tissue, is quite suppressed in *Triglochin*, free nuclei only being formed, and then only in some cases.

The mature embryo-sac is oval in shape, and frequently contains the normal number of nuclei.

The chief interest of this structure lies in the fact that the antipodal cells exhibit a fairly considerable range of variation as regards the number which may be produced. Whilst in some instances the number may be as low as three, in others a tissue may be formed such as has been described as occurring in many of the lower Monocotyledons, e. g. Grasses (9 and 10), *Sparganium* (1 *a* and 1 *b*), *Lysichiton* (1 *a*), and some other Araceae (1 *d*). The number of antipodals thus formed is never so large as occurs, according to Campbell, in *Sparganium simplex*, in which plant 150 have been observed. As a matter of fact the largest number of antipodal cells counted in *Triglochin* was in the preparations illustrated by Figs. 28 and 29, and in this instance fourteen nuclei were made out. These numbers are similar to those obtained by Hofmeister (9) for the antipodals of Triticeae. It is an interesting fact that the embryo-sac which possessed fourteen antipodal cells also showed other peculiarities, for the number of cells observed at the micropylar end numbered five, and these cells were all of an equally large size, and similar in appearance (see Figs. 28-31, which represent preparations of the same embryo-

sac). Other embryo-sacs which showed a multiplication of the antipodal cells were quite normal as regards their egg-apparatus. Preparations showing the actual divisions of the antipodal cells were unfortunately not obtained, so that it is impossible to say whether they increase by fragmentation or by karyokinesis.

A considerable amount of time was spent in endeavouring to obtain preparations at the time of actual fertilization, in order to ascertain whether the same phenomena obtain in *Triglochin* as have been found in *Lilium* by Nawaschin (12) and Guignard (7). These efforts were unfortunately unsuccessful, and the failure may perhaps be at least partly due to the small size of the nuclei concerned.

The polar nuclei, as far as could be ascertained without the actual stage of fertilization of the oosphere having been observed, do not appear to fuse till after fertilization, and, if this be correct, *Triglochin* in this respect would differ from *Sparganium simplex*, and would resemble *S. Greenii* as described by Campbell.

Examples of the fusion of these polar nuclei are illustrated in Figs. 30 and 32, Pl. VII.

#### EMBRYOLOGY.

The embryology of *Triglochin maritimum* in its chief features conforms to the type commonly met with amongst most Monocotyledons.

The chief peculiarities which distinguish *Triglochin* lie in the earlier divisions of the oospore.

The first division of the oospore takes place by means of a transverse wall cutting the original cell into a lower basal and a terminal embryo-cell (Fig. 34).

The terminal embryo-cell then divides by another transverse wall, thus giving rise to a three-celled structure (Fig. 35).

The next division which takes place was not actually observed, only the four-celled embryo which is thus produced being seen (Fig. 36), but judging from appearances it seems

extremely probable that the four-celled embryo is formed by a transverse division of the central cell of the previous three-celled stage.

The terminal cell next divides by a longitudinal wall which is frequently somewhat oblique; thus there is formed a five-celled embryo (Fig. 37).

Up to this point the divisions which have taken place in the oospore of *Triglochin* are identical with those which have been described as occurring in *Alisma Plantago* and *Sagittaria variabilis* by Schaffner (13 a and 13 b), and also with those of the oospore of *Sparganium* as described by Campbell (1 b).

The embryology of *Triglochin* differs from that of *Lilaea subulata*, H.B.K., as investigated by Campbell (1 c), inasmuch as in *Lilaea* the first longitudinal division occurs before the four superposed cells are formed.

The divisions which next occur may vary somewhat, as two distinct types were seen.

It sometimes happens that the two terminal cells of the embryo may again divide, before any other division takes place, by longitudinal walls, thus giving rise to a group of four terminal cells in the same plane. Such a case is illustrated in Fig. 38. When this is the case, the next division occurs in the cell immediately below the terminal ones, by means of a longitudinal wall (Fig. 38).

In the second type of division the cell directly below the terminal embryo-cell divides before any further divisions take place: this seems to be the commoner sequence, and when such is the case the next division is a longitudinal one occurring in one of the two terminal cells; this division is followed by a similar one in that cell which is diagonally opposite to the cell just divided in the tier above. Very frequently, however, and immediately before this takes place, it happens that the cell next above the basal cell divides by a transverse wall, so that a tier of five cells, including the basal cell, results (Fig. 39). Divisions now go on rapidly, resulting in the formation of an embryo such as that illustrated in Fig. 41, Pl. VII. Up to this stage it is possible to



distinguish the primary segment-walls, but in later stages this becomes increasingly difficult.

By subsequent growth in length the spherical embryo is converted into one of a more oval shape, as illustrated in Fig. 42. From this figure it may be seen that the basal cell is still attached to the embryo, and that there is a narrow chain of suspensor-cells.

The basal cell becomes detached from the embryo, but may be recognized for some time, until the embryo is well on its way to maturity. The stem-apex just arising is shown in Fig. 42, and from this it will be seen that, as in so many other Monocotyledons, the stem-apex arises laterally.

The mature embryo is straight, and not curved over, as is found in *Alisma Plantago*, and a second leaf is frequently differentiated while still in the seed.

#### THE FORMATION OF THE ENDOSPERM.

The first division of the definitive nucleus was observed in an embryo-sac in which the oosphere had been fertilized (Fig. 33); the two nuclei thus formed were found to be in a state of division when the embryo was a two-celled structure (Fig. 34).

The endosperm-nuclei formed are very distinct, each having a conspicuous and somewhat large nucleolus. These nuclei are not very numerous, considering the size of the embryo-sac, and they arrange themselves in the protoplasm which lines its walls. In no case were cell-walls observed between them, *Triglochin* thus differing from the endosperm of *Sparganium* and *Lysichiton*, and resembling that of *Lilaea subulata*.



SUMMARY.

*Structure.*

(a) *Rhizome.*

1. The endodermis is very similar to that of the roots of *Dracaena*, &c.
2. The vascular bundles are of the concentric type, with the xylem surrounding the phloem and broken up into separate masses by passage-cells. The protoxylem is placed towards the centre of the axis.
3. The course of the bundles is of the ordinary Palm-type, although a large number of anastomoses takes place. *Réseaux radicipères* are formed.
4. Cambium may be developed, which may be indicative of incipient secondary thickening.

(b) *Roots.*

1. The roots are adventitious, and arise acropetally.
2. A well-marked exodermis is formed.
3. The vascular cylinder may be pentarch, hexarch, or heptarch.
4. Each phloem-group is reduced to one sieve-tube, which borders directly upon the endodermis; the companion-cell is easily recognized.

(c) *Leaves.*

1. The vascular bundles are collateral.
2. In the axils of the leaves gland-like bodies are developed, and are finally cut off by a thickening of the cells across the base of each.

The glands seem to have a mucilaginous secretion.

(d) *Flowering-stem.*

1. The vascular bundles are of the collateral type, and when young have a structure remarkably similar to that of the same organs in several plants belonging to the Ranunculaceae.

*Flower-development, &c.*

1. The various parts of the flower are arranged in alternate whorls of three, which arise in acropetal succession. The anterior calyx-lobe is the first organ to arise.

The ovule is basilar in origin.

2. The embryo-sac often contains an increased number of antipodal cells, the number varying from three to fourteen, as far as has been seen.

3. The polar nuclei do not appear to fuse till after fertilization has taken place.

4. The embryology follows a normal course strongly resembling that of other Monocotyledons. A basal cell is developed, and it appears that this does not undergo division.

5. The embryo does not bend over, as in *Alisma*; the stem-apex is developed laterally.

6. An endosperm is formed, the nuclei of which are not separated by means of cell-walls; they are very distinct, lining the inner wall of the embryo-sac.

The greater part of this research was carried out in the botanical laboratories at the Royal College of Science, and I should like here to express my best thanks to Professor Farmer for the help and advice he has given me.

My thanks are also due to the Director of the Royal Gardens, Kew, and to Dr. Scott, Hon. Keeper of the Jodrell Laboratory, for allowing me to work in the Jodrell Laboratory during part of the summer vacation.

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EXPLANATION OF FIGURES IN PLATES  
VI AND VII.

Illustrating Mr. Hill's paper on *Triglochin maritimum*.

Abbreviations :—*antips.*, antipodal cells; *a. s.*, air-spaces of the medulla; *a. s.<sup>l.</sup>*, air-spaces of the cortex; *a. s. l.*, air-spaces of the leaf; *asm.*, assimilatory tissue; *b. c.*, basal cell; *camb.*, cambium; *carp.*, carpel; *c. c.*, central cavity; *co.*, cortex; *comp. c.*, companion-cell; *cot.*, cotyledon; *cu.*, cuticle; *d. n.*, definite nucleus; *emb.*, embryo; *end.*, endodermis; *exod.*, exodermal-like thickening; *g.*, glandular body; *g. c.*, guard-cell; *l.*, leaf; *l. b.*, leaf-base; *osp.*, oospore; *p.*, petal; *ph.*, phloem; *p. mer.*, primary meristem; *p. n.*, polar nucleus; *pxy.*, protoxylem; *rt.*, root; *s.*, sepal; *s. a.*, stem apex; *scl.*, sclerenchyma; *s. l.*, second leaf; *s. pl.*, sieve-plate; *s. t.*, sieve-tube; *sta.*, stamen; *syn.*, synergidae; *th. par.*, thickened parenchyma; *v. b.*, vascular bundle; *xy.*, xylem.

All the figures, excepting those relating to the flower-development, were outlined by means of an Abbe Camera lucida, the details being drawn by freehand. The lenses used were made by Zeiss.

Fig. 1. Diagram of a transverse section of an old rhizome. × 45.

Fig. 2. Transverse section of a rhizome, showing the vascular bundles. × 300.

Fig. 3. Transverse section of a rhizome, showing the cambium and a young bundle. × 250.

Fig. 4. Diagram of a transverse section of a peduncle. × 82.

Fig. 5. Transverse section of a peduncle, showing a young vascular bundle with primary meristem. × 650.

Fig. 6. Similar section, showing an older bundle with the surrounding tissue lignified. × 360.

Fig. 7. Transverse section of leaves; the sheathing base of the outermost one is shown; somewhat diagrammatic. × 45.

Fig. 8. Transverse section of a leaf, showing cortical structure. × 250.

Fig. 9. Longitudinal section of a glandular body. × 160.

Fig. 10. Similar section, showing the endodermoid markings at the base. × 360.

Fig. 11. Similar section of an older gland, showing the basal cells thickened to a much greater extent. × 650.

Fig. 12. Transverse section of a root, showing the exodermis and outer cortex. × 650.

Fig. 13. Transverse section of a root, showing the general structure. × 320.

Fig. 14. Longitudinal section of a root in the outer cortex of the rhizome, showing a sieve-tube and plates. × 680.

Fig. 15. First primordium of the flower, (*a*) in surface view; (*b*) from the side.

Fig. 16. Young flower, showing the first sepal and the second one just arising.

Fig. 17. Similar preparation of a slightly older flower seen from the side.

Fig. 18. A flower with all three sepals formed.

Fig. 19. A similar preparation of an older flower, showing the first two petals.

Fig. 20. An older flower with all the perianth-lobes complete.



Fig. 21. A flower with the outer staminal whorl complete, and showing the first signs of the inner whorl at the base of  $p^1$ .

Fig. 22. A flower with the staminal whorl complete.

Fig. 23. Part of a flower, showing the horse-shoe-shaped primordium of a carpel.

Fig. 24. A longitudinal section of a young carpel, showing the basilar nature of the ovule.

Fig. 25. A somewhat older carpel seen from above.

Fig. 26. Longitudinal section of a slightly older carpel, showing the bending over of the outer wall and the development of the stigmatic surface.

Fig. 27. A complete flower but immature.

Fig. 28. Longitudinal section of an ovule, showing numerous antipodal cells.  $\times 680$ .

Fig. 29. The next section, showing more antipodal cells.  $\times 680$ .

Fig. 30. The micropylar end of the same embryo-sac, showing two cells of the egg-apparatus.  $\times 360$ .

Fig. 31. The next section, showing three more cells of the egg-apparatus.  $\times 360$ .

Fig. 32. Embryo-sac in longitudinal section, showing the fusion of the polar nuclei.

Fig. 33. Longitudinal section of an embryo-sac, showing the first division of the embryo-sac nucleus. The synergidae are disintegrating.  $\times 360$ .

Fig. 34. Longitudinal section of an embryo-sac, showing the two daughter-nuclei of the embryo-sac nucleus dividing, and also a two-celled embryo.  $\times 360$ .

Fig. 35. Longitudinal section of a three-celled embryo, the basal cell slightly collapsed.  $\times 650$ .

Fig. 36. Similar section of a four-celled embryo.  $\times 650$ .

Fig. 37. Similar section of a five-celled embryo.  $\times 650$ .

Fig. 38. Similar section of a seven-celled embryo, the cell beneath the terminal cell undergoing division.  $\times 650$ .

Fig. 39. Diagram of a longitudinal section of a young embryo.

A. The first section, showing two terminal cells.

B. The next section, showing one terminal cell.

C. Plan of the topmost tier of cells.

C'. Plan of the second tier of cells.

Fig. 40. A. Longitudinal section of a nine-celled embryo.  $\times 650$ .

B. Plan of terminal cells.

C. Plan of tier immediately below.

Fig. 41. Longitudinal section of a young embryo. Cell-contents not indicated.  $\times 650$ .

Fig. 42. A similar section of an older embryo. The stem-apex is just arising; suspensor-cells also shown. Somewhat diagrammatic.  $\times 160$ .

Fig. 43. Similar section of a mature embryo, showing the stem-apex with a second leaf and also the primary root.  $\times 45$ .

Fig. 44. Longitudinal section of a somewhat younger embryo, showing the stem-apex and the primary root.

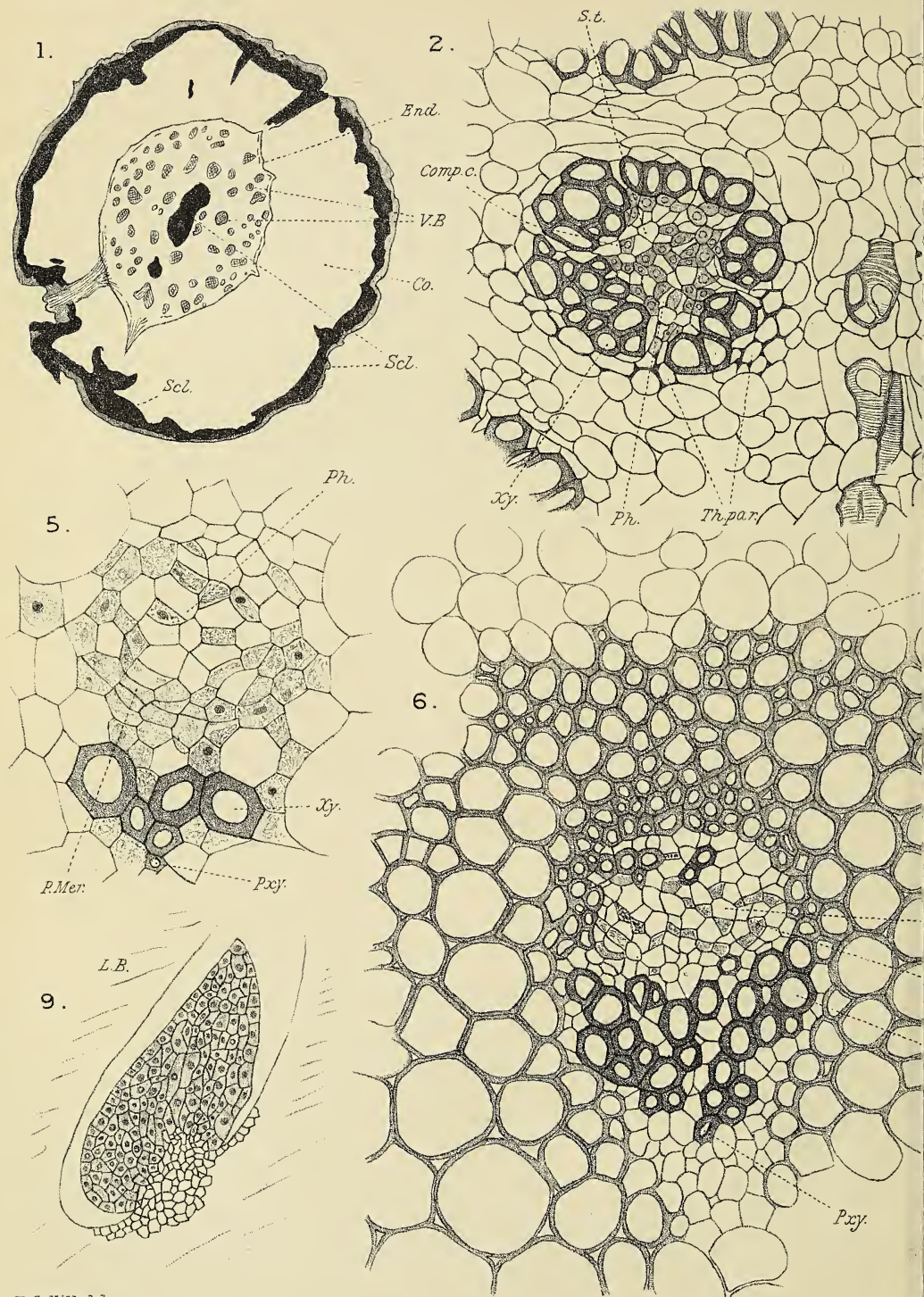
Fig. 45. A. Diagram of a transverse section of the sheathing-base of a leaf.

B. Similar diagram of the upper region of the same leaf.

The corresponding numbers in each refer to the same bundles. The letters in diagram B refer to bundles which were not found in the sheathing-base of the leaf.

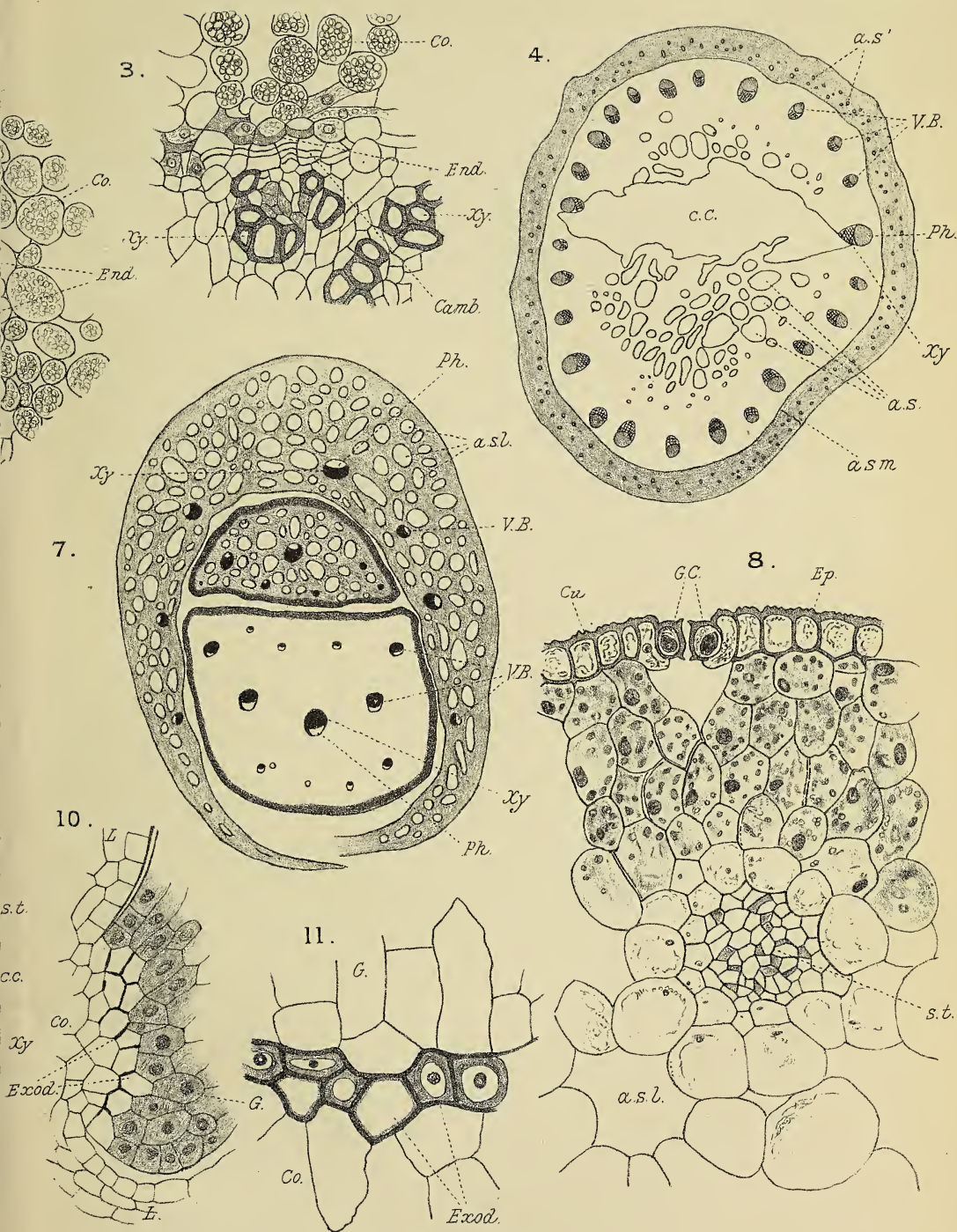






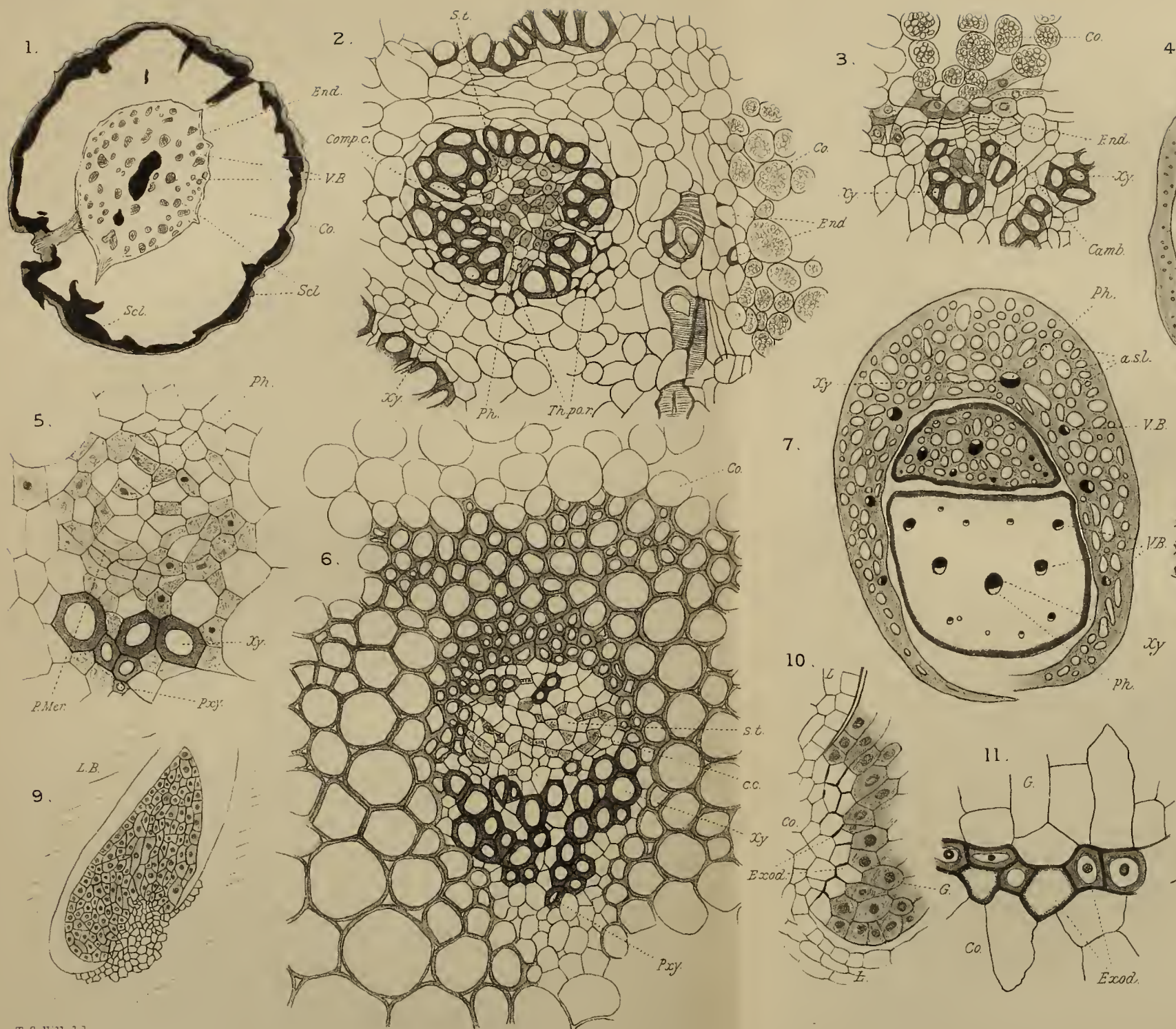
T. G. Hill, del.











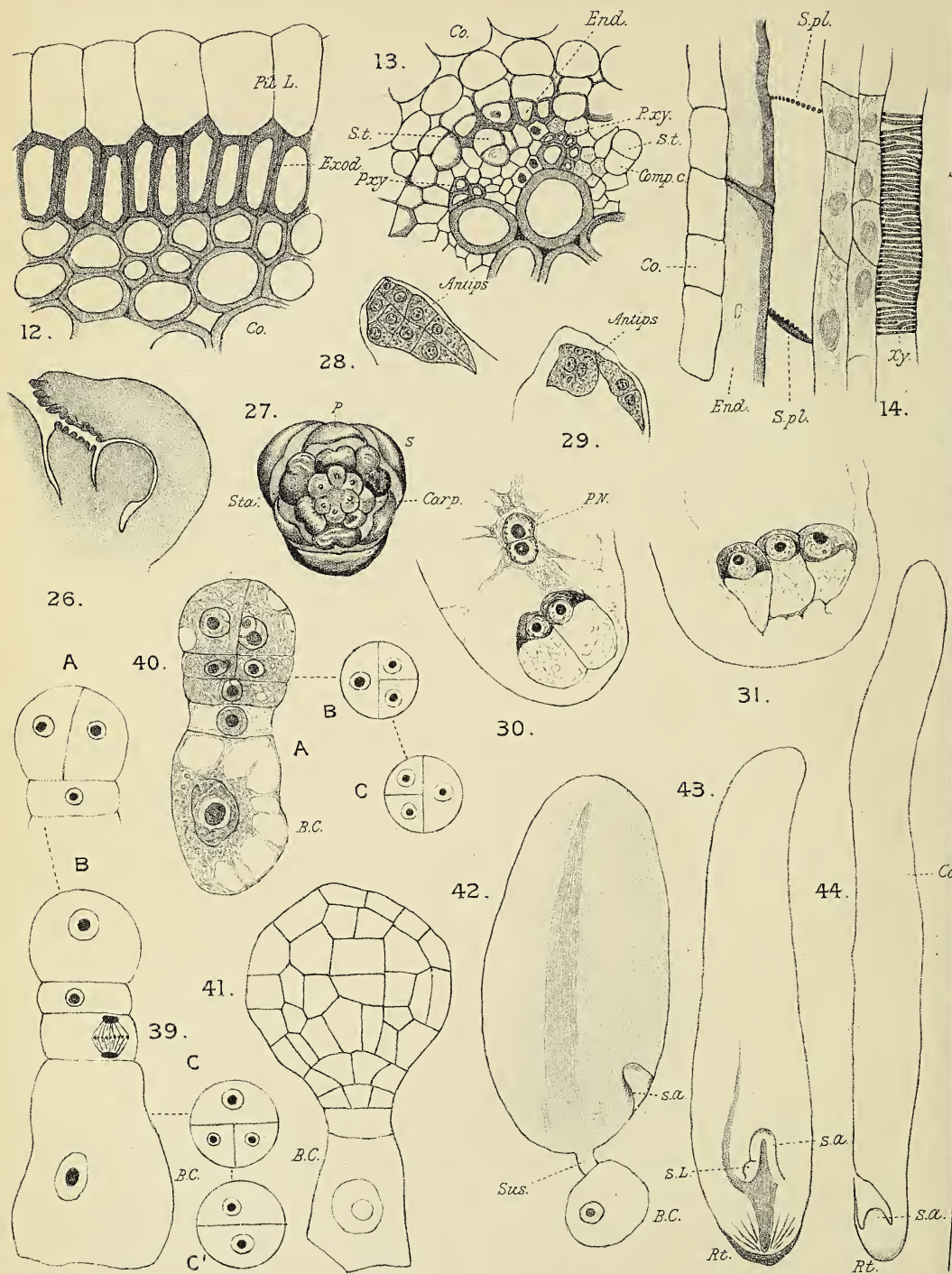
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HILL. — ON TRIGLOCHIN.









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# The Maidenhair Tree (*Ginkgo biloba*, L.).

BY

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AND

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With Plates VIII—X.



## INTRODUCTORY.

AMONG living plants there is perhaps no more striking example of a genus which recalls the past than the Maidenhair tree of China and Japan, a type of vegetation almost unknown in a wild state, but carefully preserved in the far East as a sacred tree in the gardens of temples, and frequently cultivated in Europe and America for decorative purposes. *Ginkgo* is sometimes spoken of as unknown in a wild condition, but this statement has recently been challenged by Mrs. Bishop (Miss Bird)<sup>1</sup>, who speaks of having ‘met with several fine specimens in the magnificent forests which surround the sources of the Gold River and the smaller Min in Western China.’ The same writer, in her book entitled ‘Unbeaten Tracts in Japan<sup>2</sup>,’ describes a *Ginkgo* tree in the Lebungé valley, which ‘at a height of three feet from the ground, divides into light lofty stems, none of them less than two

<sup>1</sup> Letter to the Standard, Aug. 17, 1899.

<sup>2</sup> Bird ('80), vol. ii, p. 144.

[*Annals of Botany*, Vol. XIV. No. LIII. March, 1900.]

feet five inches in diameter.' A recent writer in the *Gardeners' Chronicle*<sup>1</sup> expresses his opinion that Dr. Henry also met with the Maidenhair tree in a wild state in South-West China.

The photograph reproduced in Plate VIII is taken from a water-colour sketch executed by a native artist under the direction of Mr. Robert Fortune during his residence in China. Our cordial thanks are due to Mrs. Robb for the generous loan of the original picture, which is of interest as representing the habit of a well-grown tree from the point of view of a Chinese artist. Several photographs of fine examples of *Ginkgo* trees grown in English gardens have been published from time to time in the *Gardeners' Chronicle*<sup>2</sup>.

The single existing species has long been recognized as a member of the Gymnosperms possessing certain features suggestive of remote antiquity, and nearly related to species which were widely distributed during the Mesozoic and Tertiary eras. While usually included in the Taxineae, *Ginkgo* has for many years been regarded as a peculiar generic type exhibiting numerous points of contact with the Cycadaceae, and the most cogent reason for giving full expression to its isolated position has been supplied by Hirase's discovery<sup>3</sup> of ciliated antherozoids, a striking confirmation of Hofmeister's view<sup>4</sup> that these motile male elements would probably be found to be developed in the pollen-tubes of Gymnosperms.

A new subdivision of the Gymnospermae, the Ginkgoaceae, has now been adopted by Engler<sup>5</sup> and others for the reception of the monotypic genus *Ginkgo*, distinguished from the true Coniferae by the possession of motile male cells as well as by other characters of more or less importance. While sharing with the Cycads several characteristics, *Ginkgo* possesses some features which suggest comparison with the Ferns. The anatomical investigation of Palaeozoic and Meso-

<sup>1</sup> *Gardeners' Chronicle* ('99), p. 467.

<sup>2</sup> Vide the above paper for references.

<sup>3</sup> Hirase ('97), ('98).

<sup>4</sup> Hofmeister ('63), p. 438.

<sup>5</sup> Engler and Prantl ('97), p. 19.



zoic plants has conclusively demonstrated that Cycads and Ferns become more and more intimately associated as we descend the geologic series: we are led to the conclusion that these two classes of plants are the descendants of some common stock of remote antiquity. Our object in this contribution is to give a general account of the external features and internal structure of *Ginkgo biloba*, to summarize the scattered references which bear directly on the systematic position of the genus, and to present such evidence as is available towards a more accurate knowledge of the past history of the Ginkgoaceae.

The material used in our investigations was obtained chiefly from the gardens of Montpellier and Cambridge; the specimens of male flowers borne on the large Ginkgo tree in the Royal Gardens, Kew, were kindly given to us by Dr. Scott. Our thanks are due to M. Jules Daveau for female flowers produced in the Montpellier Gardens, and to Mr. Lynch for rendering us much valuable assistance in procuring material.

### HISTORICAL SKETCH.

The Maidenhair tree is first mentioned under the name *Ginkgo* in 1712 by Kaempfer<sup>1</sup>, who gives a drawing of a shoot and an ovule and speaks of the plant as 'Ginkgo or Ginan, *vulgo* Itsjo<sup>2</sup>—arbor nuci fera folio adiantino.' Kaempfer's term *Ginkgo* was adopted by Linnaeus<sup>3</sup> in his 'Mantissa Plantarum' of 1771. Thunberg<sup>4</sup> places *Ginkgo biloba*, L., among 'plantae obscurae,' and a few years later Smith<sup>5</sup> refers it to the Coniferae, substituting the designation *Salisburia adiantifolia* for the 'uncouth generic name *Ginkgo* and the incorrect specific term *biloba*.'

In 1812 Gouan<sup>6</sup> contributed a paper on *Ginkgo*, in which he figured a shoot and a male flower; he retained the older

<sup>1</sup> Kaempfer (1712), p. 811; vide also Kaempfer (1727), vol. i, p. 116.

<sup>2</sup> Vide Matsumura ('84), p. 86. *Ginkgo biloba* = Ichō and Ginan-no-ki.

<sup>3</sup> Linnaeus (1771), p. 313.

<sup>4</sup> Thunberg (1784), p. 358.

<sup>5</sup> Smith (1797).

<sup>6</sup> Gouan ('12).

generic name on the ground that Smith's reasons for the substitution of *Salisburia* were inadequate. Gouan describes a tree, received from Sir Joseph Banks, which had been growing for twenty-four years in his garden at Montpellier, where it bore male flowers in 1812; the author, in speaking of previous accounts of the Maidenhair tree, writes, 'plusieurs auteurs ont parlé de cet arbre, la plupart n'ont répété que ce qui avait été déjà dit.' In 1819 Jacquin<sup>1</sup> published an account of *Ginkgo*, illustrated by a coloured drawing of the leaves and male flowers; he mentions the production of seeds on a tree grown in Vienna. Watson<sup>2</sup> in 1825 also figured and described the leaves and male flowers, noting that a male plant flowered at Kew on May 8, 1824. In the following year Richard<sup>3</sup> published good drawings of both male and female flowers and placed *Ginkgo* among the Taxineae. In a pamphlet by Bunge<sup>4</sup>, who had been sent by the Russian Court to Pekin, *Ginkgo* is included in the Amnataceae and spoken of as 'rarior in hortis et prope templa buddhaica . . . pulcherrima et procerissima arbor . . . talem arborem vetustissimam cujus historia usque ad tempora Dynastiae Juan refertur.' A drawing of a deeply incised leaf is given by Lindley and Hutton<sup>5</sup> in their 'Fossil Flora' for comparison with *Sphenophyllum*, a Palaeozoic genus belonging to an extinct subdivision of the Pteridophytes. Endlicher<sup>6</sup> follows Richard in the inclusion of *Ginkgo* among the Taxineae, and refers to the tree as indigenous in China and cultivated in Japan; he describes the leaves of the seedling as deeply cut, and notes the frequent occurrence of two or more embryos in one seed.

A paper by Zuccarini<sup>7</sup> in 1840 marks an advance on previous accounts in the comparison instituted between *Ginkgo* and the South African Cycad *Encephalartos horridus* as regards the form of the young leaves. In addition to

<sup>1</sup> Jacquin ('19), p. 5.

<sup>2</sup> Watson ('25), vol. ii, p. 168.

<sup>3</sup> Richard ('26), p. 133.

<sup>4</sup> Bunge ('31), p. 62.

<sup>5</sup> Lindley and Hutton ('33), vol. i, Pl. XXVII.

<sup>6</sup> Endlicher ('36), p. 261; also ('47), p. 286.

<sup>7</sup> Zuccarini ('40).

figures of mature leaves and short shoots, this author gives drawings of partially expanded leaves, and draws attention to the resemblance between the short shoots of the Maidenhair tree and the stems of Cycads. Two years later a brief account of *Ginkgo* was given by Spach<sup>1</sup>, who speaks of the flowers being produced before the unfolding of the leaves. Robert Fortune<sup>2</sup>, in his 'Wanderings in the Northern Provinces of China,' writes: 'The only tree which I met with of very large size in this district (Shanghai) is the *Salisburia adiantifolia*, commonly called the Maidenhair tree, from the resemblance its leaves bear to a Fern of that name. This is one of the plants which the Chinese are fond of dwarfing, and it is consequently often seen in that state in their gardens.' In a later work Fortune<sup>3</sup> speaks of *Salisburia* as common in the neighbourhood of temples, and describes one temple where 'two noble trees guard the entrance, and one of them is the largest specimen of the kind I have met with; its circumference about 6 ft. from the ground is 28 ft., and it probably reaches a height of 100 ft.' In an important contribution by Eichler<sup>4</sup> on the Morphology of Gymnosperm flowers to Martius' 'Flora Brasiliensis,' *Ginkgo* is placed in a separate tribe Salisburieae. Henckel and Hochstetter<sup>5</sup> in their 'Synopsis der Nadelhölzer' speak of *Ginkgo* as reaching a height of 80-100 ft.; these authors institute two garden varieties, var. *variegata*, characterized by the occurrence of yellowish stripes or spots on the leaves, and var. *macrophylla*, with large lobed leaves. Nelson<sup>6</sup>, writing in 1866 under the *nom de plume* 'Senilis,' refers to the Maidenhair tree as *Pterophyllus Salisburiensis* or Salisbury's allied Pine, and mentions the garden varieties *aurea*, *argentea*, *laciniata*, *macrophylla*, and *microphylla*. Carrière<sup>7</sup> considers the origin of *Ginkgo* obscure, and remarks that the tree is met

<sup>1</sup> Spach ('42), p. 298; vide also Göppert ('50), Pl. XLIX.

<sup>2</sup> Fortune ('47), p. 129.

<sup>3</sup> Ibid. ('63), p. 129.

<sup>4</sup> Eichler in Martius ('52), vol. iv, pp. 409 and 445; vide also Saporta ('84), p. 252.

<sup>5</sup> Henckel and Hochstetter ('65), p. 373.

<sup>6</sup> Nelson ('66), p. 163.

<sup>7</sup> Carrière ('67), p. 712.



with only in a cultivated state, having been introduced into Europe in 1754; he alludes to the variety *variegata*, and calls the large-leaved form var. *laciniata*. Siebold's 'Flora Japonica'<sup>1</sup> includes a description of *Ginkgo*, accompanied by a plate; the author speaks of the tree as native in Northern China, but not in Japan—'ubi praeterlapsis saeculis adnexam tradunt.'

In the more recent botanical literature we find frequent references to *Ginkgo*, dealing more especially with its anatomical structure and with the morphology of the female flower.

### DESCRIPTIVE.

*Ginkgo biloba*, Linnaeus.

[Mantissa Plantarum, p. 313, 1771.]

1797. *Salisburia adiantifolia*, Smith, Trans. Linn. Soc. vol. iii, p. 330.

1866. *Pterophyllus Salisburiensis*, Nelson, Pinaceae, p. 163.

### *Diagnosis.*

A tree of pyramidal form reaching a height of over thirty metres, with a smooth grey bark; comparatively hardy<sup>2</sup>, and not readily killed by cold or by a smoky atmosphere; characterized among existing Gymnosperms by its flat broad leaves, deciduous in the autumn, consisting of a long and slender petiole slightly grooved on the upper surface and a lamina with venation of the *Cyclopteridis* type, varying considerably in size and shape, occasionally fan-shaped and entire, but more frequently divided into two halves by a more or less deep median division, or subdivided into several wedge-shaped lobes. The foliage leaves occur either scattered on long shoots or crowded at the apex of short shoots; the latter form of leaf-bearing axis often passes by apical growth into the

<sup>1</sup> Siebold ('70), p. 72, Pl. CXXXVI.

<sup>2</sup> Nicholson ('92), p. 34; vide also Gardeners' Chronicle, Dec. 23, 1899, p. 467.



long shoots bearing scattered leaves with a phyllotaxis of two-fifths.

Flowers dioecious. The male flowers, which occur in the axil of scale-leaves, have the form of a stalked central axis bearing scattered loosely disposed stamens; each stamen consists of a slender filament terminating in a very small apical scale, and usually two, sometimes three or four, elliptical pollen-sacs which open by longitudinal dehiscence. The pollen-grains develop a rudimentary prothallus consisting of a few cells, and before fertilization two large spirally coiled multiciliate spermatozoids are produced from the generative nuclei in the pollen-tube. The female flowers usually have the form of a long peduncle bearing two terminal elliptical ovules enclosed at the base by a collar-like envelope representing a reduced carpellary leaf. Abnormal female flowers, possessing more than two ovules, are not infrequently met with. Each ovule consists of a nucellus enclosed by a single integument, which in the ripe seed forms a thick fleshy covering surrounding a hard woody shell; the nucellus possesses a well-marked pollen-chamber, and in the mature ovule the greater part of the nucellar tissue is reduced to a thin papery layer enclosing a large embryo-sac which usually contains two archegonia. After fertilization, which may occur either before or after the ovule has fallen from the tree, the egg-cell develops directly into an embryo with two cotyledons.

The secondary wood of *Ginkgo* is composed of tracheids with numerous bordered pits on the radial and not uncommonly on the tangential walls. Resin ducts occur in abundance, both in the pith and in the cortical tissues.

## I. LEAVES.

### A. *Cotyledons*.

The embryo shown in Pl. IX, Fig. 44, removed from the endosperm of a germinating seed, illustrates the unequal length of the two cotyledons. The smaller seed-leaf is divided into two lobes by a slit extending through about half its length,

and the larger leaf is also slightly bilobed. The cotyledons appear to be united at the apex, but each retains a distinct epidermal layer, so that they are not strictly connate. In the upper part of the cotyledons there are two small vascular bundles, one in each lobe, containing three to four xylem-elements; below the point of splitting each cotyledon is traversed by a single arc-shaped bundle consisting of about nine tracheids. In the peripheral part of the cotyledons secretory canals are seen in process of development. The stalk of a cotyledon is crescent-shaped in transverse section, and is traversed by a single vascular bundle of similar form. The epidermis is well defined, the outer walls being slightly thickened; numerous secretory canals occur in the ground-tissue, and are especially abundant on the convex side of the petiole. As Worsdell<sup>1</sup> has shown, the vascular bundle is mesarch in structure; the xylem-elements are disposed in fairly regular radial rows, separated by comparatively broad medullary-ray cells; the protophloem forms a peripheral band of crushed tissue and a few secretory cells occur immediately beyond it. Several elements of centripetal xylem are met with on the inner side of the protoxylem. Transfusion-tracheids are few in number, but occasionally a single clump may be noticed on one side of the bundle.

*Ginkgo* has been described as peculiar among Conifers in possessing hypogeal cotyledons<sup>2</sup>.

#### B. *Scale-leaves.*

Plate IX, Figs. 31–36. The outermost scale-leaves from the terminal bud of a long shoot consist in the upper portion entirely of suberized tissue; in the next scale-leaves three or four peripheral layers are composed of cork cells, and in some of the bud-scales the elements of the cork-phellogen become themselves suberized<sup>3</sup>. Two vascular bundles are present in each bud-scale, containing several centripetal xylem-elements; a few transfusion-tracheids occur on each side of the

<sup>1</sup> Worsdell ('97), p. 305.

<sup>2</sup> Tubeuf ('91), p. 96.

<sup>3</sup> Cf. Haberlandt ('96), p. 123.

bundle, and on the centripetal side of the protoxylem an arc of secretory cells extends round the protophloem, and three or four secretory sacs traverse the ground-tissue. The scale-leaves represented in Figs. 31-36, taken from the bud of a seedling 20 cm. in height, illustrate a gradual transition from a small triangular scale, of which the tissues are almost completely suberized, to longer scale-leaves, in which the cork tissue is confined to the apex, passing gradually into still longer and narrower leaves, in which a small emarginate lamina is differentiated from a broad and flat stalk. The expanded lower portions of the scale-leaves bear numerous hairs, which form a distinct woolly fringe to the petiole and are abundant on the small lamina. The enlarged drawings in Figs. 39 and 40 show more clearly the conspicuous hairs on the broad petiole and small laminar portion of a scale-leaf. Somewhat similar hairs, but less strongly developed, occur on the cotyledons of *Pinus Pinea*, L.

### C. Foliage-leaves.

The manner of development of the foliage-leaves has been dealt with by Fankhauser<sup>1</sup> in a paper published in 1882. He describes the first appearance of a leaf as a transverse protuberance embracing about two-fifths of the circumference of the stem-apex; this swelling soon exhibits a distinct emargination which becomes a fairly deep median incision. The lamina is bent over the apex, and its margin is strongly in-rolled; for a time the growth is marginal, but this is succeeded by intercalary meristematic activity. As Fankhauser pointed out, the abundance of long trichomes affords an interesting peculiarity of young *Ginkgo* leaves<sup>2</sup>: on some of the small scale-leaves hairs are particularly abundant, and a few occur also on the lamina of the young foliage-leaves, but they are more numerous on the leaf-stalk, which is at first almost covered with them, especially on the inner face; in older leaves the hairs are confined to the base of the petiole, and are visible

<sup>1</sup> Fankhauser ('82), p. 5.

<sup>2</sup> Ibid. ('82), p. 7, Figs. 16-18, 22 and 23.



to the naked eye as a white downy patch. The downy hairs of the young leaves suggest a comparison with the woolly scale-leaves and young fronds of some Cycads. The older hairs may reach a considerable length, and become multicellular, consisting in the basal portion of two rows of cells containing numerous chloroplasts; these long and frequently branched filaments are attached to a slight elevation of the leaf-tissues. In the younger leaves the twisted and tangled hairs are unicellular like those of recent Cycads, and without chloroplasts. Fig. 37, Pl. IX, represents a young leaf showing a deep median incision in the lamina, and the partially inrolled margin at *a, a*; the short lines on the drawing of the lamina indicate the presence of hairs. In Fig. 39, Pl. IX, a very young foliage-leaf is shown, bearing numerous hairs, and having a deeply lobed and strongly folded lamina; Fig. 41 represents an older leaf in which the hairs form a distinct patch, *h*, at the base of the petiole. The long multicellular branched hairs in Fig. 56, Pl. X, are from the edge of the petiole of a scale-leaf possessing a small lamina.

A drawing of a small seedling *Ginkgo* has been published by Masters<sup>1</sup> in his paper on the comparative morphology of the Coniferae, which illustrates the deeply incised and lobed form of the lamina characteristic of the leaves of a seedling and of the vigorous long shoots of a mature plant.

The following notes are based on the examination of a seedling measuring 8.5 cm. in length above the cotyledons:—The buds were visible in the axils of the cotyledons; the first green leaves are sub-opposite, and 2 cm. above the seed; the lamina of the lowest is obcuneate, and traversed by a few forked veins and short secretory canals; in the second leaf there is no definite distinction between blade and stalk. The third foliage-leaf occurs 3.2 cm. higher on the stem; it is deeply bilobed, and each lobe is cut into three smaller segments. The fourth leaf is also deeply bilobed, and the two lobes are coarsely toothed; the petiole and lamina are more sharply differentiated than in the third leaf.

<sup>1</sup> Masters ('91), p. 242.



The leaves of *Ginkgo* are of considerable importance as evidence of the occurrence of the genus in former periods of the earth's history; their characteristic shape and venation render them more trustworthy than most leaves as aids in the identification of fossils. Reference is made by the earliest writers on *Ginkgo* to the Fern-like form of the leaves, which are aptly compared with the leaves of some species of *Adiantum*. Among existing Gymnosperms there are no species of which the leaves bear more than a distant resemblance to those of the Maidenhair tree. Among Angiosperms, an Australian plant, *Hakea Baxteri*<sup>1</sup>, R. Br., possesses a leaf similar in form to that of *Ginkgo*, but easily distinguished by the venation. It is to the fronds of Ferns that the leaves of *Ginkgo* approximate most closely; the reniform fronds of such species as *Adiantum reniforme*, L.<sup>2</sup>, *A Parishii*, Hook.<sup>3</sup>, *Trichomanes reniforme*, Forst., *Lindsaya reniformis*, Dry.<sup>4</sup>, *Pterozonium* (*Gymnogramme*) *reniforme*, Mart.<sup>4</sup>, present a fairly close agreement with the leaves of the Maidenhair tree, but the characteristic leaf of the latter, with its more or less deep median incision<sup>5</sup>, and less distinctly cordate base, may usually be readily distinguished from the fronds of Ferns. In Pl. IX, Fig. 30, half of a frond of *Scolopendrium nigripes*, Hook.<sup>6</sup>, is represented; this illustrates a type of leaf very different from that of the British species and very similar to *Ginkgo*, from which it differs in the anastomosing veins. A leaf of similar form has been figured by Clarke as *Scolopendrium Delavayi*, Franck<sup>7</sup>.

The leaf of *Ginkgo*, as shown in Figs. 62-66 and 70, is characterized by the long and slender petiole and the broadly obcuneate lamina; the upper margin is usually somewhat uneven and irregular in outline, the base may be either

<sup>1</sup> Hooker, W. J. ('42), Pl. CDXXXIX and CDXL.

<sup>2</sup> Ibid. ('46), Pl. LXXI A.

<sup>3</sup> Beddome ('65), Pl. XVI.

<sup>4</sup> Engler and Prantl, Nat. Pflanz., Polypodiaceae, p. 256, 1899.

<sup>5</sup> Cohn ('96), p. 130, quotes some lines written by Goethe on the bilobed leaf of *Ginkgo*.

<sup>6</sup> Hooker, W. J. ('57), Pl. IX; vide also Watson ('98).

<sup>7</sup> Clarke ('90), p. 93, Pl. XLI.

almost horizontal or more or less steeply inclined towards the petiole; the venation is of the *Cyclopteridis* type, the lowest vein in the right and left half of the lamina follows a course parallel to the edge, and gives off branches which fork repeatedly as they spread in a palmate manner towards the upper margin of the lamina<sup>1</sup>. The short secretory canals, which have their origin in the forks of the veins, are often clearly marked as short dark lines traversing the mesophyll. These canals are occasionally visible in fossil *Ginkgo* leaves, and by one author have been described as patches of a Fungus<sup>2</sup>. A striking feature of *Ginkgo* leaves is the variation in their size and shape, a fact insufficiently recognized by palaeobotanical writers<sup>3</sup>. The deeply cut lamina with more than two long and narrow segments (Figs. 63 and 66) is met with more particularly in the large leaves of vigorous long shoots; between this type and the small entire leaves characteristic of flowering shoots the difference is considerable. An unusually large bilobed leaf obtained from a tree growing in the temperate house of the Glasgow Botanic Garden measured 20 cm. across, and a still larger leaf, 21 cm. broad, is figured under the name *Salisburia adiantifolia* var. *macrophylla laciniata* in the tenth volume of the 'Flore des Serres'<sup>4</sup>. The few examples of leaf variation represented in Pl. X, Figs. 63–67 and 71, are chosen principally in illustration of the resemblance between *Ginkgo biloba* and various Mesozoic species of the genus. More than one writer has called attention to the resemblance between the more deeply lobed leaves on the vigorous shoots of *Ginkgo* and certain Mesozoic forms; Bailey, for example, speaks of such leaves as the 'fitful recollections of an ancient state'<sup>5</sup>.

A study of the anatomy of *Ginkgo* leaves reveals some features of interest from the point of view of comparison with Cycads and Conifers. The epidermis consists of cells

<sup>1</sup> Drude in Schenk ('79), vol. i, p. 654; Bertrand ('81), p. 178, Fig. 129.

<sup>2</sup> Massalongo ('59), Pl. I, Fig. 1, p. 87.

<sup>3</sup> Gardner ('83), p. 45.

<sup>4</sup> Flore des Serres ('54), p. 119.

<sup>5</sup> Bailey ('96), p. 97; vide also Ettingshausen ('90), Pl. VII.

of irregular outline, those of the upper surface being slightly larger than those of the lower; the cuticle is well marked but not very thick, and the stomata, with their guard-cells somewhat below the level of the epidermis, are confined to the lower face of the lamina. Bertrand<sup>1</sup> states that there is no palisade tissue in the mesophyll; this is true of the smaller leaves on the flowering shoots, but the cells next the upper epidermis of the larger leaves are distinctly elongated at right angles to the surface. These palisade elements are rather irregular in form and often lobed, as are also the smaller mesophyll cells; the latter are usually elongated parallel to the leaf surface, and separated from one another by numerous intercellular spaces. Short canals occur between the veins, and a group of secretory cells is found above and below each vascular bundle, which may be in contact with the phloem, but are often separated from the xylem by one or more layers of parenchyma. The xylem is formed of about a dozen tracheids, which may occur either as one group or as several smaller strands separated by medullary rays; the latter appears to be the more usual arrangement in leaves from a fruiting branch, and the former in the ordinary foliage leaves. As Worsdell<sup>2</sup> points out, the centripetal xylem is considerably reduced, and is represented by one or two tracheids. On either side of a bundle there are often one or two transfusion-elements having the form of reticulate tracheids of larger diameter than the ordinary xylem-tracheids. Some of the medullary-ray cells may be enlarged and serve as secretory elements; and a few parenchymatous cells containing crystals are present in the neighbourhood of each vascular bundle. There is no sclerenchymatous tissue, and no thick-walled hypoderm.

The petiole is traversed by two vascular bundles inclined towards one another, the xylem being made up of regular rows of pitted tracheids, numerous medullary rays, and a few centripetal elements; the transfusion-tracheids vary in number

<sup>1</sup> Bertrand ('74), p. 30.

<sup>2</sup> Worsdell ('97), p. 306; vide also Zimmermann ('80), p. 5.



and may be absent. In longitudinal section the spiral protoxylem-tracheids may be traced gradually into short and broad reticulated elements occupying a centripetal position. The centrifugal tracheids next the protoxylem are reticulately pitted, and these are succeeded by tracheids with alternate or crowded bordered pits.

## II. FLOWERS.

### *a. Female Flowers.*

The ordinary type of female flower has the form of a long naked peduncle bearing a single ovule on either side of the apex (Pl. IX, Fig. 6), the base of each ovule being enclosed by a small collar-like rim, the nature of which has been variously interpreted by different writers.

A young ovule of *Ginkgo* consists of a conical nucellus surrounded by a single integument (Fig. 47, *i*), terminating in the form of a two-lipped micropyle. A large pollen-chamber (*pc*, Fig. 47)<sup>1</sup> occupies the apex of the nucellus, and immediately below this two or more archegonia are developed at the summit of the embryo-sac, each of which consists of a large egg-cell surmounted by two neck-cells and a ventral canal-cell, which is cut off shortly before fertilization. In the young ovule shown in median longitudinal section in Pl. IX, Fig. 47, the embryo-sac is represented at *e* in an early stage of development. Pax<sup>2</sup> calls attention to the many-celled archesporium of *Ginkgo* as a character resembling the Cycads. At a later stage, after the pollen-grains have entered the pollen-chamber, the opening of the latter becomes closed, the chamber being roofed over by a blunt protuberance of nucellar tissue, and at the beginning of June it increases in size and forms a large irregularly shaped cavity. During the growth of the embryo-sac the nucellus is gradually destroyed, and early in August a vertical outgrowth is formed from the top

<sup>1</sup> Bertrand ('78).

<sup>2</sup> Pax ('90), p. 274.



of the embryo-sac, which appears as a column supporting at its apex the nucellar protuberance.

It is noteworthy that the cells surrounding the ovum are characterized by thick and deeply stained walls, and contain abundant contents and large nuclei; the walls of these cells are penetrated by well-defined pits, as in some Cycads<sup>1</sup> and Conifers. The drawing shown in Fig. 48, Pl. IX, illustrates the appearance presented in an apical view of the endosperm taken from a seed which had fallen from the tree; two archeogonia are seen in an oval depression, one on each side of the vertical outgrowth of the embryo-sac. Before fertilization<sup>2</sup> the nucleus of the egg-cell takes up a position just below the neck-cells, and a canal-cell is cut off early in September; the nucleus then moves towards the ovum and fertilization occurs. Fertilization is followed by a division of the oospore nucleus into two; each daughter-nucleus divides repeatedly, and eventually cell-membranes are laid down. The formation of the cell-walls is succeeded by division of the cells, growth being particularly vigorous in the lower part of the oospore, which constitutes the vegetative part of the embryo and gradually becomes differentiated, early in October, into the two cotyledons<sup>3</sup>. It has often been stated that fertilization does not take place until the ripe ovules have fallen from the tree; this point has recently been investigated by Hirase<sup>4</sup>, who finds that some seeds at least contain a more or less developed embryo while still attached to the parent plant.

The ripe ovule of *Ginkgo* is enclosed by a thick fleshy integument, succeeded internally by a woody envelope; the upper part of the endosperm, *d*, after the removal of the hard shell (Fig. 45, *b*, Pl. IX), is covered by a thin papery membrane, *c*, which represents the crushed remains of the nucellus. There is a close resemblance between the seed shown in Fig. 45 and a seed of a Cycad; in each the crushed nucellar tissue is represented by a thin membrane between the woody portion

<sup>1</sup> Treub. ('84); Ikeno ('98).

<sup>2</sup> Hirase ('95), ('98).

<sup>3</sup> Strasburger ('72), p. 312.

<sup>4</sup> Hirase ('94). We are indebted to Dr. Donald MacAlister for a translation from Japanese of Hirase's paper.

of the integument and the endosperm<sup>1</sup>. Fig. 43 represents the endosperm of a fallen seed cut longitudinally; in the centre there is the blunt papilla already described, on one side of which the egg-cell of an archegonium has remained undeveloped, *a*, while on the other an embryo, *a'*, has been produced.

The thick fleshy portion of the *Ginkgo* seed is rich in secretory canals, but does not possess vascular bundles; the histological structure of the seed-coats has been described by Bertrand<sup>2</sup>. The embryo-sac is usually two-angled, and the archegonia, as described by Strasburger<sup>3</sup>, occur on a line joining the two keels of the woody part of the integument. Penzig<sup>4</sup> speaks of the occurrence of three-angled seeds, which he compares with seeds of Cycads<sup>5</sup>. It is of interest to notice the similarity between the structure of *Ginkgo* seeds and some of the Gymnospermous seeds described by Brongniart<sup>6</sup> and others from Palaeozoic rocks.

The morphology of the female flower has been variously interpreted by botanists; the opinions expressed on this question, as Čelakovský says, constitute 'ein lehrreiches Capitel wissenschaftlichen Suchens und Irrrens<sup>7</sup>.' The peduncle of the flower arises in the axil of a leaf, and usually bears a single ovule (Fig. 6) on either side of the apex. Strasburger in 1872<sup>8</sup> described the ring or collar at the base of each ovule as the rudiment of the first pair of leaves of a secondary shoot; in 1879<sup>9</sup> he spoke of the same structure as an arillus, and regarded the fleshy covering of the ovule as an integument, the peduncle with its two ovules constituting an inflorescence bearing two flowers. Strasburger described

<sup>1</sup> Cf. Bertrand ('78), p. 700.

<sup>2</sup> Ibid., loc. cit.

<sup>3</sup> Strasburger ('72), p. 291.

<sup>4</sup> Penzig ('94), p. 515. Vide also Saprota ('84), p. 166, and Saprota and Marion ('85), p. 58.

<sup>5</sup> Braun ('75).

<sup>6</sup> Brongniart ('81); Hooker and Binney ('55), p. 153.

<sup>7</sup> Čelakovský ('90), p. 2.

<sup>9</sup> Ibid. ('79), pp. 74 and 120.

<sup>8</sup> Strasburger ('72).

examples in which the peduncle bore four seeds, each supported on a slender stalk instead of two almost sessile ovules<sup>1</sup>. Eichler<sup>2</sup> criticized these views, and looked upon the fleshy part of the seed as an inner integument, and the collar at the base of the ovule as the outer integument. The same author, writing in 1889<sup>3</sup>, speaks of the collar as a rudimentary carpel and the peduncle and its two ovules as a single flower. Van Tieghem<sup>4</sup> also considers the peduncle and ovules to be a single flower, the peduncle or flower-stalk being homologous with the petiole of a foliage-leaf and the two ovules comparable to the two lobes of a typical *Ginkgo* leaf; flowers bearing more than two ovules he compares with multilobed leaves. Van Tieghem describes the vascular system of the peduncle and its subtending leaf, and regards the collar of each ovule as a rudimentary arillus. According to Čelakovský the peduncle is a shoot bearing two or more carpels, each carpel being much reduced and transformed in its terminal portion into an ovule. He considers the peduncle with two ovules to be a reduced form, and looks upon the abnormal examples, in which one stalk bears more than two ovules, as valuable indications of the former existence of a type of *Ginkgo* flower which normally bore several ovules<sup>5</sup>. A Japanese author has recently summarized the chief views on the morphology of the female flower, and has himself contributed important evidence towards the solution of this vexed question<sup>6</sup>. He speaks of the peduncle as a shoot bearing two rudimentary carpels, and usually characterized by the suppression of the apical bud. Fujii adduces some interesting evidence in support of his views derived from a study of abnormal flowers in which ovules occur on more or less modified foliage-leaves (Fig. 61, Pl. X); he found

<sup>1</sup> Strasburger ('79); vide also Göppert ('50), Pl. xlix, Fig. 5; Loudon ('75), Fig. 1758; Saporta and Marion ('85), p. 139.

<sup>2</sup> Eichler ('73).

<sup>3</sup> Ibid. ('89).

<sup>4</sup> Van Tieghem ('69), ('91), p. 1460.

<sup>5</sup> Čelakovský ('90), p. 43. Vide postea, p. 145.

<sup>6</sup> Fujii ('96); Potonié (98), p. 285, has recently suggested a comparison between the female flower of *Ginkgo* and the leaf of *Botrychium*.



examples of seeds produced on the marginal portions of the lamina (Fig. 61), and in some specimens the blade of the leaf was almost entirely transformed into a group of ovules of smaller size than those borne on normal flowers. The collar at the base of the ovules was found to pass gradually into the lamina of the modified leaf. In one example figured by this author, the peduncle, which is unusually thick and bears several ovules, terminates in a scaly bud. He describes the peduncle as a shoot, and the slender stalk of each ovule is regarded as the petiole of a carpellary leaf. He found that a peduncle bearing several ovules is usually traversed by as many vascular bundles as there are ovules; each of the bundles in the peduncle divides into two in the ovule-stalks, so that each carpellary petiole possesses two small strands similar to those in an ordinary leaf-stalk.

Our own examination of normal and abnormal flowers leads us to adopt the view that the peduncle of the female flower of *Ginkgo* is a shoot bearing two or more carpels. Each ovule is enclosed at the base by an envelope or collar homologous with the lamina of a leaf; the fleshy and hard coats of the nucellus constitute a single integument. The stalk of an ovule, which is considerably reduced in the normal flower and much longer in some abnormal flowers, is homologous with a leaf-stalk, with which it agrees in the structure and number of the vascular bundles. The following examples afford evidence favourable to this view of the morphology of the female flower.

We attach considerable importance to the evidence afforded by abnormal flowers; deviations from the normal must be dealt with cautiously as aids to morphological interpretation, but granting the truth of the saying 'On verrait en elles tout ce qu'on voudrait y voir,' abnormalities are at least useful guides as to possible lines of evolution.

Pl. IX, Figs. 4, 15-20. Fig. 4, Pl. IX, represents the apex of a short shoot bearing a small foliage-leaf, shown in the drawing as a leaf-scar, *l*, subtending two slender stalks, each of which terminates in a small ovule. In a transverse section



cut below the point at which the petiole becomes free from the pedicels of the ovules the vascular tissue assumes the form of a ring of bundles separated by wide medullary rays; at a slightly higher level the ring becomes wider and forms an ellipse, and from this several tracheids pass off to form the trace of the subtending leaf, which consists of two separate collateral bundles, as in the petiole of an ordinary foliage-leaf. The remaining bundles are now arranged in two groups of two each (Fig. 15), with two rudimentary additional bundles, *r, r*, consisting of radially disposed phloem-elements, but very little xylem; these die out at a higher level (Figs. 16 and 17). A section through the base of the pedicels at the level of their separation (Fig. 18) reveals the existence of a small bud, *b*, between the ovuliferous stalks, the inner faces of which are clothed with hairs. This small bud explains the presence of the two rudimentary bundles, and may be regarded as the aborted apex of the flowering shoot which bore the two pedicels and their ovules as lateral members. In each pedicel there are two collateral vascular strands inclined towards one another, as in the double leaf-trace of an ordinary petiole; the centripetal xylem-tracheids and transfusion-elements are however more abundant in the pedicel bundles. Fig. 19 represents a transverse section of one of the pedicels; it shows two vascular bundles and two large canals; in a section of the same pedicel later at a higher level the two bundles have coalesced to form a single strand (Fig. 20), which is accompanied by numerous transfusion-tracheids and several centripetal xylem-elements.

Pl. IX, Figs. 21-23. In the ordinary female flower in which a flowering axis occurs in the axil of a leaf and bears two laterally placed ovules at the apex, the subtending leaf and the peduncle cohere at the base. A transverse section of the coherent petiole and peduncle shows the arrangement of bundles represented in Fig. 21; the lower part, *l*, passes up into the leaf-stalk and the upper part, *f*, becomes the peduncle. The leaf-stalk possesses the usual pair of bundles and the flower-stalk has four bundles in two pairs (Fig. 22); a short

distance below the ovules (1–2 mm.) one pair of bundles unites (Fig. 23), and at a slightly higher level the remaining pair become fused into a single strand. Large canals traverse the ground-tissue of the peduncle, and transfusion-tracheids, which increase in number in the neighbourhood of the ovules, occur in association with the bundles of the flower-stalk.

Pl. IX, Figs. 2 and 3, 11–14. Figs. 2 and 3 illustrate an abnormal form of flower in which the peduncle bears three stalked ovules and a lateral unexpanded bud, *b*, seen in side view in Fig. 3. Near the base of the peduncle (Fig. 11) there are two pairs of bundles and one larger separate vascular strand, five in all; at a higher level the bundles are disposed as three pairs, and a new smaller bundle (Fig. 12, *a*) is inserted between two of the pairs. Each vascular strand contains one or two centripetal tracheids, and some transfusion-elements are present. A second additional bundle is met with at a still higher level (Fig. 13), the centre of the axis being now occupied by a secretory canal, *c*. Still higher, we find that the first additional bundle has become elongated tangentially, and is accompanied by numerous transfusion-tracheids and some centripetal xylem-elements; both of the additional bundles have moved nearer the centre of the peduncle. Fig. 14 shows the arrangement of the tissue at a higher level; the additional bundles form a broken ring surrounding the central canal, *c*, and these strands supply the lateral bud. Each of the three pairs of peripheral bundles passes into an ovuliferous stalk where the double strand unites into an arc-shaped bundle. The lateral bud (*b*, Figs. 2 and 3) shows three small protuberances, which in section present the appearance of rudimentary ovules.

Pl. IX, Figs. 1 and 5. In the first example (Fig. 1) the stout peduncle of a female flower bears five ovuliferous stalks, each of which is traversed by a pair of bundles. Fig. 5 represents a peduncle bearing two sessile lateral ovules at the apex, and at a lower level three small ovules on slender pedicels.

*β. Male Flowers.*

The male flower<sup>1</sup> has the form of a catkin borne in the axil of a scale-leaf on a short shoot, with one or more bracts (Fig. 8, *b*) attached to the peduncle<sup>2</sup>. Usually two (Fig. 9), but not infrequently three, and more rarely four pollen-sacs (Fig. 7) depend from a slender filament terminating distally in a small knob, which appears as a broader fleshy disc, bearing four pollen-sacs in the stamen shown in Fig. 7. The pollen-sacs dehisce by a longitudinal slit on the inner side (Fig. 9). The wall of the sac consists of four to seven cells in breadth, and thickening bands occur on the walls of the outer layers. Thibout<sup>3</sup> has recently given an account of the structure of the pollen-sacs and filament; he suggests that the stamens with three pollen-sacs may point to the former existence of a type of *Ginkgo* stamen in which three was the normal number. The pollen-grains ( $30\ \mu \times 10\ \mu$ ) are characterized by a median depression along their major axis, and as Thibout points out they recall those of Cycads rather than the pollen-grains of Conifers<sup>4</sup>.

The peduncle of the male flower adheres at its base to the subtending bract; in a section through the common base the vascular bundle of the peduncle is semicircular in form, and the two bundles of the bract complete the circle. In the free peduncle the xylem consists of nine or ten separate strands arranged in the form of an ellipse; there appears to be little or no centripetal xylem or transfusion tracheids. The filament of a stamen is traversed by an axial bundle composed of about twelve tracheids radiating from a protoxylem strand (Fig. 50, *px*); two or three centripetal xylem-elements occur (cf. Fig. 50), also a few large transfusion-tracheids (*to*).

At maturity—about the end of April—a pollen-grain con-

<sup>1</sup> Goebel ('81), p. 705 (Pl. VI, Figs. 25 and 26), has described the development of the stamens of *Ginkgo*.

<sup>2</sup> Van Tieghem ('91).

<sup>3</sup> Thibout ('96), p. 175.

<sup>4</sup> Thibout ('96), p. 199. Vide also Capellini and Solms ('92), Pl. V; Wieland ('99), p. 389.



tains a prothallus of three to five cells and the exine embraces two-thirds of the circumference. The pollen-tube grows towards the top of the nucellus, and at the beginning of July the distal end of the tube becomes branched and fixes itself near the apical nucellar protuberance, the pollen-grain end being next the archegonia. The nucleus of the innermost prothallus-cell divides, and one daughter-nucleus is pushed out from the mother-cell; this cell ('body-cell' of Strasburger) grows in size and reaches its maximum about the middle of August. Some days before fertilization the cell divides, and finally two large antherozoids are produced<sup>1</sup>.

### III. SHOOT AND ROOT<sup>2</sup>.

#### i. *Leaf-traces*.

The course of the leaf-traces in shoots of *Ginkgo* has been described by Thomas<sup>3</sup>, Geyler<sup>4</sup>, and other writers. Close to the apex of a shoot<sup>5</sup> the vascular bundles of a foliage-leaf make their appearance as double strands, and the leaf-traces in the upper part of the shoot have the form of distinct bundles which in the lower region constitute a continuous ring. Each double leaf-trace passes through four internodes before becoming a part of the stele; the double form of the trace is especially characteristic of *Ginkgo*<sup>6</sup>. The first indication of the separation of a leaf-trace from the stele of the shoot, as seen in transverse section, is the intrusion of two blunt wedges

<sup>1</sup> Hirase ('94), p. 360, and ('98); Strasburger ('92), Pl. I; Belajeff ('97), p. 338; Webber ('97).

<sup>2</sup> For references to the anatomy of *Ginkgo*, vide Mohl ('32), p. 411; Hartig ('48), p. 123; Göppert ('50), pp. 54 et seq., Pls. IX and XIII; Dippel ('62) and ('63); Kraus ('64), pp. 146, &c., and ('86), pp. 102, &c.; Van Tieghem ('70), p. 186; Bertrand ('74); Höhnelt ('77), p. 535; Moeller ('82), Fig. 21; Nakamura ('83), p. 25; Gardner ('83); Göppert ('83); Schulz ('83); Saporta ('84), pp. 20 et seq.; Essner ('86), pp. 10, &c.; Felix ('94).

<sup>3</sup> Thomas ('65).

<sup>4</sup> Geyler ('67); vide also Nägeli [quoted by Bertrand ('74)].

<sup>5</sup> Strasburger ('72), p. 327; vide also Fankhauser ('82).

<sup>6</sup> Williamson ('83), Pl. XXXIII, Figs. 28 and 29.



of the pith into the xylem-ring (Fig. 24, *p, p*); a third smaller wedge (Fig. 25, *p'*) makes its appearance between the two larger ones, and this rapidly extends in a radial direction until the vascular cylinder is broken through (Fig. 26); finally, the two groups of xylem and phloem<sup>1</sup> become free (Fig. 27), and pass outwards (Fig. 53, *lt*) into the leaf-stalk accompanied by one or more large secretory canals (*S*). Close to the base of the leaf-lamina each bundle divides into two and then breaks up into the dichotomously branched veins.

ii. *Young stem (seedling).*

A transverse section of a young stem 2.5 mm. in diameter presents the following features:—The epidermis is cuticularized, but no cork has been formed in the hypodermal tissues; the xylem occurs as a ring made up of groups separated by wide medullary rays, each group being composed of radial rows of tracheids and medullary rays one to two cells in width; the phloem is approximately equal to the xylem in depth, and the crushed protophloem is succeeded by secretory cells. The protoxylem elements are often compressed and separated from the radially disposed tracheids by one or two layers of parenchyma. Long secretory sacs occur in the pith and canals traverse the cortex; the cortical region also includes scattered cells containing crystals of calcium oxalate.

iii. *Short and long Shoots.*

The type of stem represented by a seedling is also met with in the terminal elongating shoots of the adult tree; the phyllotaxis of these long shoots may be  $\frac{2}{5}$ ,  $\frac{3}{8}$ , or  $\frac{5}{13}$ ; each leaf bears in its axil a bud which in the third year often develops into a short shoot producing a few crowded leaves. The short shoot elongates from year to year and bears an apical group of leaves, the older portion being covered with the crowded leaf-scars of former years, presenting an appearance

<sup>1</sup> Cf. *Cordaitea* (*Dadoxylon*) as figured by Williamson ('77), Pl. IX, pp. 44 and 46.

comparable on a small scale (Fig. 42) to the main stem of a Cycad. A short shoot may, after several years' growth, elongate into a long shoot<sup>1</sup> bearing scattered leaves, and in some instances the short shoot may branch (Fig. 42), like the trunks of *Cycas* and some other genera of Cycads.

In the long shoot the xylem and phloem form a complete ring; secretory canals traverse the tissues of both pith and cortex, and secretory cells are especially abundant at the periphery of the phloem. The medullary rays of young long shoots were found to be usually one cell deep, rarely two or three cells in depth. The tracheids bear one to three rows of bordered pits on their radial walls, and when more than one row is present the pits of adjacent series may occur on the same level; single rows of pits are fairly abundant on the tangential walls of the xylem-elements.

In comparing a series of sections cut through the upper part of a short shoot and the lower portion of the long shoot into which it has elongated, one notices certain differences in the structure. The wood of the short shoot is rather looser in texture (Fig. 58), more particularly at the inner margin of the xylem, where the rows of tracheids are separated by broad medullary rays as in Cycadean wood (Figs. 53 and 58). In the cortex of a short shoot the phloem is succeeded by ordinary parenchyma, including crystal-sacs; in the long shoots a broken ring of thick-walled and crushed secretory cells succeeds the phloem, and farther out crystal-sacs are abundant in the parenchymatous tissue. Canals are met with in the pith and cortex of both long and short shoots. The pith-cells of the short shoots are more or less spherical and irregularly disposed, while in the long shoots they are more rectangular and in regular vertical series. With the exception of certain species of *Cephalotaxus*, as recently pointed out by Rothert<sup>2</sup>, secretory canals are not found in the pith of true Conifers. In the short shoots the phloem is characterized by the occurrence of thick-walled fibres. Reticulately pitted tracheids are abundant at the point of exit of a leaf-trace, and

<sup>1</sup> Bertrand ('74), p. 24.

<sup>2</sup> Rothert ('99).

a few short elements of this type may occur on the centripetal side of the foliar strand.

The drawing shown in Fig. 59 represents the tracheids of a leaf-trace as seen in a tangential section through the cortex of a short shoot; the smaller group, *x*, may belong to an axillary bud. In Fig. 10 a short shoot is seen in radial longitudinal section; the contraction of the pith has produced an appearance suggestive of a discoid pith like that of the Walnut or the extinct genus *Cordaïtes*.

#### iv. Older branches.

There are a few points in the structure of older branches worthy of note. The secondary phloem contains numerous thick-walled fibres with transverse or oblique cross-walls; with these are associated parenchymatous cells and large sieve-tubes with sieve-plates in small groups on the radial walls (Fig. 52). These several elements do not follow a regular or constant order of succession in a radial direction, but are variously arranged. In the secondary bast single swollen parenchymatous cells occur full of crystals; similar sacs are found also in the cortex, pith, and medullary-ray tissues. In the autumn wood bordered pits are abundant on the tangential walls of the tracheids (Fig. 49), the pit-canals are often twisted, and appear as a cross when seen in surface-view. The bordered pits on the radial walls of the tracheids are often separated from one another by transverse radial bars<sup>1</sup>. The medullary rays are two to five cells deep, and their cells have simple oblique pits, but in some cases these appear to be bordered; the branched medullary-ray cells of an old branch shown in Fig. 57 are unusual in form, and pursue a somewhat obliquely radial course<sup>2</sup> across the face of the tracheids.

Strasburger<sup>3</sup> has described the anatomy of a *Ginkgo* stem fifty-eight years old; it is unnecessary therefore to recapitulate the facts he records.

<sup>1</sup> Strasburger ('82), p. 42, Pl. III.

<sup>2</sup> Cf. Russow ('83).

<sup>3</sup> Strasburger ('92), p. 45.



v. *Roots.*

Van Tieghem<sup>1</sup> and other writers have described some of the anatomical features of the roots of *Ginkgo*. Fig. 51, Pl. X, represents a section of a young primary root; the piliferous layer produces long unicellular hairs, of which traces can be seen in the photograph; this is succeeded by one or two layers of rectangular suberized cells and four layers of cortical elements; next to this tissue there are two layers, or sometimes only one layer, of cells with strong thickening bands on their radial walls (Fig. 51, *e*). The pericycle is made up of seven to eight layers of almost spherical cells, but opposite the two xylem plates this tissue is narrower. A radial longitudinal section through the place of origin of a lateral root demonstrates the existence of several short tracheids with bordered pits between the spiral protoxylem and the tracheids of the lateral root. The spiral tracheids of the protoxylem are succeeded by elements with reticulate pitting, and beyond which there are tracheids with large transversely elongated simple pits which gradually pass into the elements with smaller bordered pits<sup>2</sup>.

In the seedling root the stele is at first diarch; but at a higher level the splitting of first one and then the other xylem strand produces a tetrarch structure; at a still higher level a fifth protoxylem group appears, and afterwards a sixth is found on the opposite side of the stele, thus producing an hexagonal arrangement (Pl. X, Fig. 55). Scattered xylem-elements (metaxylem<sup>3</sup>) appear in the pith, and these may be abundant enough to entirely replace the central conjunctive tissue. In the hypocotyl the cambium forms rows of centrifugal xylem except at the two ends of the stele, the latter gradually increases in diameter, and from the two ends of the longer axis of the xylem-ring, groups of tracheids pass out as the cotyledon-traces; the stele then becomes closed again,

<sup>1</sup> Van Tieghem ('70), p. 195; ('87), p. 105; also Van Tieghem and Douliot ('88), p. 349, and Strasburger ('72), p. 350.

<sup>2</sup> Cf. Dippel ('62), Pl. VI, Fig. 1.

<sup>3</sup> Van Tieghem ('87), p. 105.



and assumes the form of a ring consisting of seven or eight collateral bundles of xylem and phloem.

In a fairly old root the annual rings are not so well marked as in the stem, and the walls of the tracheids are thinner; the medullary rays which contain a few crystal-sacs vary in height from one to seven cells. The xylem parenchyma includes some crystal-containing cells, and thick bast fibres are abundant in the phloem.

### FOSSIL GINKGOACEAE.

To attempt a detailed description of the fossil records which have a direct or indirect bearing on the geological history of the Ginkgoaceae would necessitate an unreasonable extension of the present paper. Our aim must be confined to giving, as briefly as possible, a general summary of the more trustworthy evidence which may enable us to form an opinion as to the antiquity of the Ginkgoaceae, and as to the geographical distribution of fossil species in former periods of the earth's history. In 1881 Oswald Heer<sup>1</sup> published an interesting article, in which he summarized the data furnished by Palaeozoic, Mesozoic, and Tertiary fossils towards the past history of the existing type. The list of plants enumerated includes certain forms which cannot be satisfactorily shown to bear a close relationship to *Ginkgo*, and, on the other hand, subsequent research enables us to make additions to Heer's list of extinct species.

*Palaeozoic leaves.* Among the genera recorded from Carboniferous and Permian rocks, which have been regarded as more or less closely related to the existing Maidenhair tree, we have *Ginkgo* itself, *Ginkgoephyllum*, *Baiera*, *Saportaea*, *Trichopitys*, *Dicranophyllum*, *Rhipidopsis*, *Whittleseya*, *Psygmo-phyllum*, *Cordaites*, *Gomphostrobus*, *Trichophyllum*, and others.

*Ginkgo (Salisburia).* Under the name *Salisburia primitiva*, Saporta and Marion<sup>2</sup> have figured some fossils described by Grand'Eury from the middle Permian of the

<sup>1</sup> Heer ('81).

<sup>2</sup> Saporta and Marion ('85), p. 145, Fig. 74.

Urals, which bear a fairly close resemblance to the leaves of the recent species. These fragments, while agreeing in shape with some types of *Ginkgo* leaves, may also be compared with a Russian fossil described by Schmalhausen<sup>1</sup> as *Rhipidopsis ginkgoides* from strata classed as Jurassic, but which Zeiller<sup>2</sup> has shown probably belong to the Permo-Carboniferous period. Renault<sup>3</sup> has figured a leaf from the Permian of Martenet, which may be compared with a specimen named by Schmalhausen *Ginkgo integerrima*<sup>4</sup>; and Archangeli<sup>5</sup> records *G. primigenia* from the Permian of Italy.

*Psygmorephyllum* (*Ginkgoephyllum*). Saprota<sup>6</sup> proposed the name *Ginkgoephyllum* for leaves of a cuneate form with an entire or dissected lamina attached to the stem by a decurrent leaf-stalk. He includes under this term leaves described by some authors<sup>7</sup> as species of *Noeggerathia*, *Psygmorephyllum*, and other genera. We have no evidence, beyond such as is afforded by their not very close resemblance to the leaves of *Ginkgo*, that these fossils have any claim to be regarded as representatives of the Ginkgoaceae.

*Saportaea*. This generic name was instituted by Fontaine and White<sup>8</sup> for two species of leaves from the Virginian Coal-Measures, which resemble those of *Ginkgo biloba*. In themselves the fossils are insufficient as evidence of the existence of the Ginkgoaceae in the Coal Period forests, but they are of interest as agreeing closely in form with those of recent species.

*Rhipidopsis*. Schmalhausen<sup>9</sup> provided this genus for some leaves with a palmate lamina and dichotomously branched veins, from Petschoraland, which he placed in the Salisburieae. The genus has been recorded more recently by Kurtz<sup>10</sup> from

<sup>1</sup> Schmalhausen ('79), Pl. VIII, Fig. 3.

<sup>2</sup> Zeiller ('96).

<sup>3</sup> Renault ('96), p. 138.

<sup>4</sup> Schmalhausen ('79), Pl. XVI.

<sup>5</sup> Archangeli ('95).

<sup>6</sup> Saprota ('84), p. 230.

<sup>7</sup> Lindley and Hutton ('32), Pls. XXVIII and XXIX; vide also Schimper ('70), p. 192; Schenk ('83), Pl. XLIII.

<sup>8</sup> Fontaine and White ('80), Pl. XXXVIII.

<sup>9</sup> Schmalhausen ('79), p. 50; vide also Zeiller ('96).

<sup>10</sup> Kurtz ('94).

Permo-Carboniferous rocks in Argentina. As in the case of the Palaeozoic species, the leaves of this genus naturally suggest a comparison with *Ginkgo*, but we have no satisfactory evidence beyond external resemblance.

*Gomphostrobus*. This genus, described by Marion<sup>1</sup> from the Permian of Lodève and placed by him in the Salisburieae, bears too remote a resemblance to *Ginkgo* to be included in the list of probable near allies of the recent species. Potonié<sup>2</sup> speaks of the genus as possibly a member of the Psilotaceae, but this opinion has little to support it.

*Dicranophyllum*. A Palaeozoic genus characterized by the possession of narrow linear forked leaves attached to leaf cushions which cover the surface of the branches<sup>3</sup>. Such evidence as we possess regarding the nature of the reproductive organs does not lend support to the views of some authors that *Dicranophyllum* should be included in the Ginkgoaceae.

*Trichopitys*. Saporta<sup>4</sup> has applied this name to Permian and Jurassic leaves characterized by a deeply dissected lamina with forked acicular segments. The type-species, *Trichopitys heteromorpha* from the Permian of Lodève, presents a fairly close resemblance to *Dicranophyllum*, but we have not sufficient evidence to warrant its inclusion in the Ginkgoaceae. A Jurassic plant, originally described by Lindley and Hutton as *Solenites furcata*<sup>5</sup>, is named by Saporta *Trichopitys Lindleyana*<sup>6</sup>; this species should probably be referred to *Baiera*, and may be a near ally of *Ginkgo*.

The genus *Cordaite*, although it cannot reasonably be classed with the Ginkgoaceae, is of interest as affording certain points of contact with *Ginkgo*<sup>7</sup> which suggest a prob-

<sup>1</sup> Marion ('90), p. 892; vide also Zeiller ('92), p. 101, Pl. XV, Fig. 12.

<sup>2</sup> Potonié ('93), p. 192, Pls. XXVII, XXVIII, and XXXIII.

<sup>3</sup> Founded by Grand'Eury ('77), p. 1021; vide Lima ('88); Renault ('96), Pls. LXXXIX and LXXXI, p. 376.

<sup>4</sup> Saporta ('84), p. 230, Pl. CLII.

<sup>5</sup> Lindley and Hutton ('37), Pl. CCIX.

<sup>6</sup> Saporta ('84), p. 266, Pl. CLV.

<sup>7</sup> Williamson ('77) and ('83) has described the double leaf-trace of *Cordaite* similar to that of *Ginkgo*; vide also Scott ('96), p. 17, who compares the stamens of *Ginkgo* and *Cordaite*.



able alliance between the two genera. The genera *Whittleseya* and *Trichophyllum* have been compared with *Ginkgo*<sup>1</sup>, but our knowledge of these fossils is too incomplete to admit of more than speculation of little or no scientific value.

*Baiera*. This genus calls for a fuller notice as including several species of Palaeozoic and Mesozoic age, some of which are almost certainly near relatives of the Maidenhair tree. *Baiera* was first defined by Braun<sup>2</sup>, who applied the name to some Triassic leaves which agree with *Ginkgo* in shape, but differ in possessing a lamina with more numerous and narrower segments. Braun and Schenk<sup>3</sup> included *Baiera* among the Ferns, and it is not improbable that some of the species may be best compared with such recent Ferns as *Actinopteris radiata*, Link. (Pl. X, Fig. 67), or with species of *Schizaea*, e.g. *S. dichotoma*, Sw. (Pl. X, Fig. 60), *S. elegans*, Sw., and others.

Valuable evidence as to the Gymnospermous nature of some types of the genus is afforded by examples of flowers and seeds described by Schenk<sup>4</sup>, Heer<sup>5</sup>, and other authors. Among Palaeozoic leaves referred to *Baiera*, we have *Baiera virginiana*, Font. and Wh.<sup>6</sup>, from the Permian of Virginia, *B. Raymondi*, Ren.<sup>7</sup>, from Charmoy, and some other species. The genus was probably most widely spread during the Jurassic period, but there is fairly strong evidence in favour of extending its range to the Palaeozoic epoch. In considering the range of *Baiera* it is important to bear in mind the absence of any well-marked distinguishing features between some species of this genus and some of the more dissected forms of *Ginkgo* leaves. Among Jurassic leaves of the *Ginkgo* type [e.g. *G. digitata* (Brongn.) and *G. Phillipsi*, Nath.] it is easy to select a series illustrating a gradual transition from leaves with an entire lamina to those with a dissected lamina and linear segments, conforming in all respects to Braun's

<sup>1</sup> Lesquereux ('79), Pl. IV ; Saporta and Marion ('85), p. 144, Fig. 73 C.

<sup>2</sup> Braun ('43), p. 20.

<sup>3</sup> Schenk ('67).

<sup>4</sup> Ibid.

<sup>5</sup> Heer ('76), p. 51.

<sup>6</sup> Fontaine and White ('80), Pl. XXXVII.

<sup>7</sup> Renault ('96), p. 138, Fig. 51.



genus *Baiera* and to leaves which some authors include in the genus *Jeanpaulia*.

*Triassic.* Among Triassic species referred to *Baiera* and *Ginkgo*, the following may be mentioned:—*Baiera multifida*, Font., from Virginia<sup>1</sup>, which bears a close resemblance to *Salisburia palmata*, Ratte, from Australia<sup>2</sup>; *Ginkgo crenata*<sup>3</sup> (Brauns), from Steinstedt near Braunschweig; *Baiera furcata*, Heer, founded on a fragment from the Swiss Trias<sup>4</sup>; *Baiera* (?) *Steinmanni*, Solms, from the Rhaetic of Chili<sup>5</sup>, similar to *B. Schenki*, Feist.<sup>6</sup>, from South Africa, and *B. Virginiana*, F. and Wh.; *Baiera Munsteriana* (Presl), *B. taeniata*, Schenk, from Germany<sup>7</sup>; *Ginkgo obovata*, Nath., *G. minuta*, Nath. (possibly a Fern; cf. *Rhacopteris* sp.), *Baiera Geinitzi*, Nath., *B. marginata*, Nath., *B. paucipartita*, Nath., from Scania<sup>8</sup>. From the Ipswich rocks of Queensland, Shirley<sup>9</sup> has recently described several species of *Ginkgo*, e.g. *G. antarctica*, Sap., *G. bidens*, Ten-woods (very similar to *Baiera gracilis*, Bunb., from the Inferior Oolite of Yorkshire), *G. Simmondsi*, Shir., and *Baiera ginkgooides*, Shir.

Some of the best examples of Rhaetic species of *Baiera* have been described by Schenk. This author also describes and figures some fossils under the name *Stachopitys Preslii*<sup>10</sup>, which are no doubt male flowers, of which the stamens bear five or six pollen-sacs arranged as a verticil at the end of the filaments.

*Jurassic and Wealden.* The genera *Ginkgo* and *Baiera*<sup>11</sup> are abundantly represented in Jurassic floras, and especially from European localities. We cannot attempt a critical examination of all the described species, but must confine ourselves to the enumeration of most of the examples recorded from various latitudes. The drawings of *Ginkgo* leaves shown in Pl. X, Figs. 62–66 and Fig. 70, illustrate the range of variation in the form of the lamina in the recent species, and serve as warnings

<sup>1</sup> Fontaine ('83), Pls. XLV–XLVII.

<sup>2</sup> Ratte ('87).

<sup>3</sup> Nathorst ('78).

<sup>4</sup> Heer ('76).

<sup>5</sup> Solms-Laubach ('99).

<sup>6</sup> Feistmantel ('89).

<sup>7</sup> Schenk ('67) and ('87).

<sup>8</sup> Nathorst ('78).

<sup>9</sup> Shirley ('98).

<sup>10</sup> Schenk ('67).

<sup>11</sup> The genus *Czekanowskia*, frequently included in the same family with *Ginkgo*, is omitted as being in all probability but remotely connected with the recent genus.

against accepting all the fossil types of Heer and other authors. The species *Ginkgo digitata*, originally described by Brongniart as a Fern<sup>1</sup>, is a common fossil in the Inferior Oolite beds of the Yorkshire coast, and one of the best examples of a Jurassic representative of the Maidenhair tree (Fig. 46, Pl. IX). The leaves of this species are in some forms practically indistinguishable from those of *Ginkgo biloba* (Figs. 46 and 54). Some of the smaller and more deeply lobed examples are usually referred to a distinct species, *Ginkgo Huttoni* (Sternb.), but these bear too near a likeness to some forms of the recent species (e.g. Figs. 63, 64, and 66, Pl. X) to be specifically separated from the more typical examples of *Ginkgo digitata* (Pl. IX, Fig. 46). The fossil leaf shown in Fig. 69, Pl. X, from the Stonesfield slate, is probably not specifically distinct from the *Ginkgo digitata* (Brongn.), but it is of interest as affording evidence of the existence of the genus in the Stonesfield slate flora, from which it has not been previously recorded. The Wealden leaves from North Germany, originally named by Dunker *Cyclopteris digitata*, Brongn.<sup>2</sup>, can hardly be distinguished from the Jurassic species.

Among other species of *Ginkgo* and *Baiera* may be mentioned *Ginkgo Whitbiensis*, Nath., *Baiera gracilis*, Bunb., *B. Phillipsi*, Nath., *B. Lindleyana* (Schimp.), all of which occur in British Oolite strata. From Northern latitudes Heer<sup>3</sup> has described numerous leaves which he refers to *Ginkgo* and *Baiera*; from Siberia we have such species as *Ginkgo pusilla*, *G. concinna* (cf. *Baiera gracilis*), *G. lepida* (cf. *Salisburia nana*, Daws.<sup>4</sup>, from Canada, *B. Phillipsi*, Nath., and *Ginkgo sibirica*, Heer), *G. sibirica*, *G. digitata*, *Baiera longifolia*, and *B. pulchella*. The specimens from Franz Josef Land described by Nathorst<sup>5</sup> and by Newton and Teall<sup>6</sup> as *Ginkgo polaris*

<sup>1</sup> Brongniart ('28), Pl. LXI bis.

<sup>2</sup> Dunker ('46), Pls. I, V, and VI; and Schenk ('71), Pl. XXIV.

<sup>3</sup> Heer ('68-'83), passim.

<sup>4</sup> Dawson ('85 and '93).

<sup>5</sup> Nathorst in Nansen ('97), Vol. ii, p. 486.

<sup>6</sup> Newton and Teall ('97), Pl. XXXVIII. [Since this was written, a fuller account of *Ginkgo* leaves from Franz Josef Land has been published by Nathorst in Part III of the Scientific Results of the Norwegian North Polar Expedition, 1893-1896, edited by Fridtjof Nansen.]

may be compared with *Ginkgo Whitbiensis* from England and with *G. pulchella* from Siberia ; they are chiefly of interest as extending the Arctic range of the genus.

From rocks which may be of Inferior Oolite age at Cape Stewart on the east coast of Greenland, Hartz<sup>1</sup> has recorded *Ginkgo Hermelii*; from Bornholm<sup>2</sup> we have *G. digitata* and *Baiera pulchella*. From China and Japan leaves of both *Ginkgo* and *Baiera* are recorded by Schenk<sup>3</sup> and Yokoyama<sup>4</sup> respectively.

Feistmantel has determined an imperfect Indian specimen on very slender evidence as *Ginkgo* sp.<sup>5</sup> The plant-beds of India have not furnished any specimens which afford satisfactory evidence of either *Baiera* or *Ginkgo* in the Permo-Carboniferous or Mesozoic floras of that region.

*Cretaceous and Tertiary.* Cretaceous rocks of North America, Greenland, and other regions have afforded several examples of *Ginkgo* leaves, such as *Ginkgo polymorpha*, *G. Laramiensis*<sup>6</sup> [very similar to *G. adiantoides* of Tertiary age ; Fig. 29, Pl. IX], *G. pusilla*, Daws., from Vancouver Island<sup>7</sup> ; *G. primordialis*, *G. multinervis* from Greenland<sup>8</sup>.

The Tertiary species of *Ginkgo* named by Unger *G. adiantoides*<sup>9</sup> presents a striking agreement with the existing type, and indeed is hardly distinguishable from it ; this species has been recorded from Italy, Siberia, Scotland (Mull), and North America. Some unusually good specimens have been described by Starkie Gardner<sup>10</sup> from the leaf-beds of the Island of Mull ; one of the Mull leaves from the British Museum Collection is shown in Fig. 29, Pl. IX ; the veins are very clearly marked, and in some examples traces of secretory canals may be recognized in the lamina as in *Ginkgo biloba*. Gardner is of opinion that 'there can be no reasonable doubt as to the specific identity of the Ardtun (Mull) fossil and the living *Ginkgo biloba*' ; he suggests the designation *G. biloba hebraidica*.

<sup>1</sup> Hartz ('96), Pl. XIX.

<sup>3</sup> Schenk ('83).

<sup>5</sup> Feistmantel ('79), Pl. XV.

<sup>7</sup> Dawson ('93), Pl. VI.

<sup>9</sup> Unger ('45), p. 211.

<sup>2</sup> Bartholin ('94), Pl. IV.

<sup>4</sup> Yokoyama ('89).

<sup>6</sup> Ward ('86), Pl. XXXI.

<sup>8</sup> Heer ('68-'83).

<sup>10</sup> Gardner ('83), Pl. XXV.



Among other Tertiary species there are *Ginkgo reniformis*, described by Heer<sup>1</sup> from Siberia; *G. borealis* from the west coast of Greenland, and *G. Laramiensis*, Ward (probably identified with *G. adiantoides*), from the Laramie beds of North America<sup>2</sup>.

*Flowers and seeds.* The existence of Palaeozoic seeds very closely allied to those of *Ginkgo* has already been noticed. There is a strong probability that some at least of the *Ginkgo*-like leaves of Palaeozoic age were borne by plants possessing no distant affinity with the recent species, but how near the relationship between the past and present types was it is impossible to decide. Our knowledge of many of the Gymnospermous seeds from Permian and Upper Carboniferous horizons is fairly complete so far as concerns their internal structure, but we have little knowledge as to the plants which bore the seeds. It would seem probable that there existed in the Permo-Carboniferous forests extinct types, such as *Cordaites* and other genera, which cannot be fitted into any of our existing families, but possessed certain characters, in either their vegetative or reproductive organs, which have persisted as characteristic features of the Maidenhair tree of to-day.

The recognition of certain characteristics of the Ginkgoaceae in Palaeozoic types does not by any means demonstrate the existence or even the probable existence of the family in Permian or Carboniferous times, but it is more in accordance with experience to expect that extinct genera of so remote an antiquity should exhibit points of affinity with more than one existing family. The plants which possessed characters nearest akin to those of *Ginkgo* were probably members of the Cordaitales, an extinct stock with which the Ginkgoaceae are closely connected.

The best examples of flowers of Mesozoic age which may reasonably be referred to plants bearing leaves of either the *Ginkgo* or *Baiera* type are those described by Heer and Schenk. Associated with the numerous *Ginkgo* leaves in

<sup>1</sup> Heer ('68-'83).

<sup>2</sup> Ward ('86), Pl. XXXI.



the Jurassic plant-beds of Siberia, Heer has found several specimens of male flowers which agree very closely with those of the recent species. From a central axis numerous loosely disposed stamens are given off at a wide angle, and the filaments bear two or sometimes three or more terminal pollen-sacs<sup>1</sup>. The specimen shown in Fig. 28, Pl. IX, somewhat enlarged, represents an imperfect specimen of a male flower from the Inferior Oolite beds of Yorkshire; it is less perfect than the Siberian examples, but of interest as the best so far recognized from an English locality. There is a striking similarity as regards the external form between the fossil and recent type of flower. A few isolated pollen-sacs of *Ginkgo* were figured by Phillips<sup>2</sup> in 1829 as 'unknown leaves'; the true nature of the fossil shown in Fig. 28 being first suggested by Nathorst.

The flowers associated with *Baiera* leaves and described by Schenk and other authors are similar to those of *Ginkgo*, but differ in the greater number of pollen-sacs borne on each stamen<sup>3</sup>.

As regards the female flowers the evidence is somewhat less conclusive. Heer has figured several fossil seeds and portions of peduncles which he refers on fairly good grounds to the genus *Ginkgo*, but for the most part the seeds occur as detached bodies, and throw little light on the nature of the female flowers of the Jurassic species of the Ginkgoaceae. The most interesting fossil from our present point of view is one which Carruthers described in 1869 under the name *Beania gracilis*<sup>4</sup>; in general structure this species is very similar to a female flower of the Cycad *Zamia*, in which the individual carpophylls are farther apart than in the recent type of cone. If we imagine the internodes of a *Zamia* flower considerably elongated, we have an arrangement closely resembling *Beania*. The central axis, which is fairly short and woody, bears loosely disposed secondary axes attached at right angles; these branches are probably carpophylls, and each

<sup>1</sup> Heer ('68-'85), passim.

<sup>2</sup> Phillips ('29), Pl. VII, Fig. 23.

<sup>3</sup> Schenk ('67).

<sup>4</sup> Carruthers ('69).

consists of a slender pedicel bearing two oval or subspherical seeds, with a fleshy outer coat, on the inner face of a peltate distal expansion. Schimper<sup>1</sup>, Carruthers, and other writers regard *Beania* as a Cycadean flower, and Potonié<sup>2</sup> prefers the generic name *Zamiostrobus* to *Beania* as more definitely expressing the affinity. Suggestions have been made by Schimper and Saporta<sup>3</sup> to account for the differences between *Beania* and typical Cycadean flowers, but these need not be discussed.

It is perhaps the most natural conclusion to draw that *Beania* was borne by one of the plants with pinnate Cycadean fronds, of which several species occur in the Lower Oolite strata. Our opinion, on the other hand, is that *Beania* does not improbably represent the female flower of a plant with leaves of the *Ginkgo* type and male flowers like that shown in Fig. 28, Pl. IX. This view seems to us to derive support from the following considerations. So far as we know, the Jurassic Cycadean plants possessed flowers of the *Bennettites* type; this has, I believe, been satisfactorily demonstrated as regards *Williamsonia gigas*<sup>4</sup>, and there is little doubt that the well-known fronds originally known by the name of *Pterophyllum pecten* were associated with the female flowers known as *Williamsonia Leckenbyi*, Nath. In short, we have evidence that the Cycadean stems from Jurassic and Cretaceous horizons bore flowers which differed considerably from those of recent Cycads; the evidence in support of this statement is derived from a study of English, Italian, French, German, and American specimens. We have indeed no satisfactory example of a Mesozoic Cycadean flower, constructed on the plan of the female cones of recent Cycads, which can be reasonably connected with a plant bearing Cycadean foliage.

The abundance of *Ginkgo* leaves in the Inferior Oolite rocks renders it probable that some trace should be found of the reproductive organs; specimens of male flowers are not very rare, and the evidence that these belong to *Ginkgo* or

<sup>1</sup> Schimper ('70), p. 206.

<sup>2</sup> Potonié ('98), p. 278, Fig. 274.

<sup>3</sup> Saporta ('75), p. 59.

<sup>4</sup> Seward ('95), p. 146, ('97).

*Baiera*, although not absolutely conclusive, is almost convincing. Heer has figured isolated seeds associated with *Ginkgo* leaves and a few specimens in which seeds appear to be attached to peduncles, but the examples are far from perfect. Detached seeds are not uncommon in the English Jurassic rocks, and these appear to be identical with those borne by the *Beania* type of flower; they are characterized by the possession of a fleshy integument which in the fossil state presents a wrinkled appearance. *Beania gracilis* is constructed on the same plan as the male flowers of *Ginkgo*, and differs from the female flowers of the recent species in the greater number of ovules and in the manner in which the ovules are attached to the peduncle. The ovules of *Beania* are attached to the inner side of the expanded end of each pedicel or carpophyll. If we imagine the ovules of *Ginkgo* turned through an angle of  $180^\circ$  we have the collar-like envelope occupying the same position as the peltate expansion in *Beania*. Some of the abnormal flowers of *Ginkgo*, such as those figured by Fujii<sup>1</sup> and that shown in Fig. 5, Pl. IX, approach more closely to the *Beania* type, and it is not improbable that these examples indicate ancestral features, as Čelakovský<sup>2</sup> has suggested. Without wishing to overstrain such arguments as may be adduced in favour of this view, we prefer to regard *Beania gracilis* as a female flower, which was more probably borne by a plant belonging to the Ginkgoaceae than by a member of the true Cycadaceae.

Some isolated seeds from the London Clay of Sheppey have been doubtfully referred to *Ginkgo* under the name *Ginkgo? eocenica*<sup>3</sup>, but these are in themselves insufficient as evidence of the existence of the genus in the Sheppey deposits.

The records of fossil wood do not materially assist us in our review of the geological history of *Ginkgo*. The Tertiary wood named by Göppert *Physematopitys Salisburioides*<sup>4</sup>, and

<sup>1</sup> Fujii ('96).

<sup>2</sup> Čelakovský ('90).

<sup>3</sup> Gardner ('83), p. 46.

<sup>4</sup> Göppert ('52), p. 270.



regarded by him as agreeing most closely with the wood of *Ginkgo*, has been re-examined by Kraus and identified as the root-wood of the *Cupressoxylon* type<sup>1</sup>. The structure of the stem of some Palaeozoic plants<sup>2</sup> exhibits points of resemblance to *Ginkgo*, but evidence of this kind leads to the same conclusion as that afforded by the Palaeozoic leaves; it demonstrates the existence of morphological features in certain synthetic or composite plant-types which are now met with in the Maidenhair tree.

### CONCLUSION.

Our examination of the floral and vegetative structures of the Maidenhair tree leads us to adopt the view that this isolated type should be placed in a separate division of the Gymnosperms—the Ginkgoaceae—and no longer included in the Coniferae.

In many respects *Ginkgo* shows a marked affinity with the Cycads; like the extinct Cycadofilices, *Ginkgo* possesses both Filicinean and Cycadean characters, but while exhibiting traces of the union of Cycads and Ferns, it represents in all probability a very ancient type which may have been merged into the Cordaitales in the Palaeozoic era. Among the numerous features in which *Ginkgo* resembles the Cycadaceae, the most striking are recognized in the ovules and seeds, in the production of spermatozoids, and in certain anatomical characters referred to in the above description of the reproductive and vegetative organs.

The records of the rocks demonstrate both the antiquity and wide geographical range of *Ginkgo* and allied forms during the Mesozoic and Tertiary periods; species of *Ginkgo* and *Baiera* have been discovered in almost all parts of the world. The majority of the older representatives of the Ginkgoaceae agree more closely with the genus *Baiera*; in the Jurassic, Cretaceous, and Tertiary periods, *Ginkgo* itself

<sup>1</sup> Kraus ('86), p. 75.

<sup>2</sup> e.g. *Dadoxylon Pedroi*, Zeill., from Permo-Carboniferous rocks of Brazil. Vide Zeiller ('95).

appears to have been especially abundant in more northern latitudes, whence it may have gradually retreated to escape from unfavourable climatic conditions.

It is sometimes stated that the more deeply-lobed leaves of the Maidenhair tree recall most vividly the ancient forms; this statement, while in the main supported by palaeontological evidence, must not be made without reserve. Leaves with an entire lamina are occasionally met with in Jurassic strata (Pl. X, Fig. 54); both forms of leaf were represented, though on the whole the species with more numerous and narrower segments predominated.

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## EXPLANATION OF FIGURES IN PLATES VIII, IX, AND X.

Illustrating the paper by Mr. Seward and Miss Gowan on the Maidenhair Tree.

### PLATE VIII.

Photograph of a water-colour painting of *Ginkgo biloba*, by a Chinese artist.  
(Photographed by Mr. Edwin Wilson from the original sketch in the possession of Mrs. Robb.)

### PLATE IX.

- Fig. 1. Abnormal female flower with five small ovules (nat. size).  
Figs. 2, 3. Abnormal female flower with three ovules and a bud *b* (nat. size).  
Fig. 4. Abnormal female flower; *b*, leaf-scar (nat. size).  
Fig. 5. Abnormal female flower (nat. size).  
Fig. 6. Female flower with two large ovules (nat. size).  
Fig. 7. Four pollen-sacs attached to one filament (slightly enlarged).  
Fig. 8. Male flower; *b*, bract (nat. size).  
Fig. 9. A single stamen showing two open pollen-sacs (slightly enlarged).  
Fig. 10. A short shoot cut in half longitudinally, showing an irregularly 'discoid pith' (slightly enlarged).  
Figs. 11-23. Diagrams illustrating the position and number of vascular bundles in the abnormal flowers shown in Figs. 1-4.  
Figs. 24-27. Diagrams illustrating the connexion between the double leaf-trace and the stele of a long shoot of *Ginkgo*. *s* = group of secretory cells.  
Fig. 28. Part of a male flower of a fossil *Ginkgo* from the Inferior Oolite of Gristhorpe Bay, near Scarborough (slightly enlarged. Leckenby collection, Cambridge).  
Fig. 29. *Ginkgo adiantoides* from the Eocene leaf-beds of Mull (nat. size. British Museum).  
Fig. 30. *Scolopendrium nigripes*. Half of a leaf, showing the veins and sori (nat. size. Botanic Garden, Cambridge).  
Figs. 31-37. Scale-leaves and young foliage-leaf from the terminal bud of a long shoot (slightly enlarged).  
Fig. 38. Two small leaves borne on the stem of a seedling (slightly enlarged).  
Figs. 39-41. Scale-leaf and young foliage-leaves. *h* = hairs (slightly enlarged).  
Fig. 42. Branched short shoot (nat. size).  
Fig. 43. The endosperm of a fallen seed in median longitudinal section.  
*a* = archegonium; *a'* = embryo (nat. size).

Fig. 44. Embryo separated from the endosperm (nat. size).

Fig. 45. Seed of *Ginkgo*, showing the woody testa *b*, the endosperm *d*, and the remains of the nucellus *c* (nat. size).

Fig. 46. *Ginkgo digitata*, from the Inferior Oolite (nat. size. Manchester Museum).

Fig. 47. A young ovule in median longitudinal section. *i* = integument; *pc* = pollen-chamber; *e* = young embryo-sac (slightly enlarged).

Fig. 48. Apical view of the endosperm of a fallen seed showing two archegonia, *a*, *a* (slightly enlarged).

#### PLATE X.

Fig. 49. Tangential section through the autumn wood of a branch.

Fig. 50. Transverse section of the filament of a stamen. *px* = protoxylem; *cp* = centripetal xylem; *ts* = transfusion tracheids.

Fig. 51. Transverse section of a young primary shoot. *e* = endodermis.

Fig. 52. Longitudinal section through the phloem of a branch.

Fig. 53. Transverse section through part of the stele of a short shoot. *lt* = double leaf-trace.

Fig. 54. *Ginkgo digitata*, from the Inferior Oolite near Scarborough (slightly reduced. York Museum).

Fig. 55. Transverse section of the root of a seedling.

Fig. 56. Multicellular hairs from the petiole of a scale-leaf.

Fig. 57. Radial longitudinal section through the secondary xylem of a branch showing tracheids and medullary-ray cells.

Fig. 58. Transverse section of a portion of the xylem of a short shoot.

Fig. 59. Tangential section through the cortex of a short shoot. *x* = group of tracheids.

Fig. 60. *Schizaea dichotoma*, Siv. (slightly enlarged. The Herbarium, Cambridge).

Fig. 61. Modified foliage-leaf of *Ginkgo biloba* bearing a marginal ovule (after Fujii).

Figs. 62-66. Leaves of *Ginkgo biloba*.

Fig. 67. *Actinopteris radiata*, Link (nat. size).

Fig. 68. *Baiera gracilis*, Bunb., from the Inferior Oolite of Yorkshire (British Museum) (nat. size).

Fig. 69. *Ginkgo* sp., from Eyeford (slightly reduced. Cirencester Museum).

Fig. 70. Leaf of *Ginkgo biloba*.

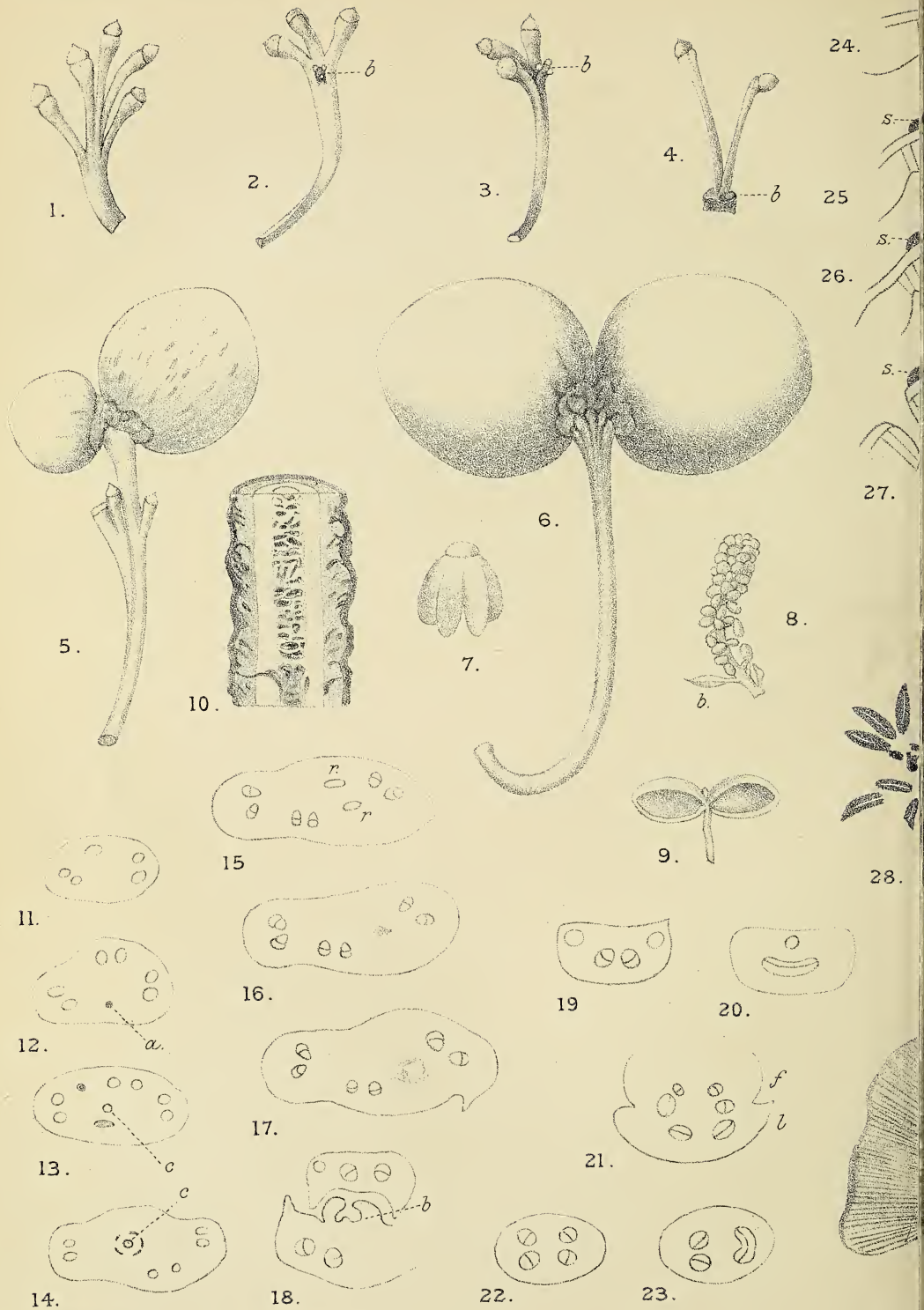












J. Gowan del.

SEWARD — ON GINKGO.  
 & GOWAN.









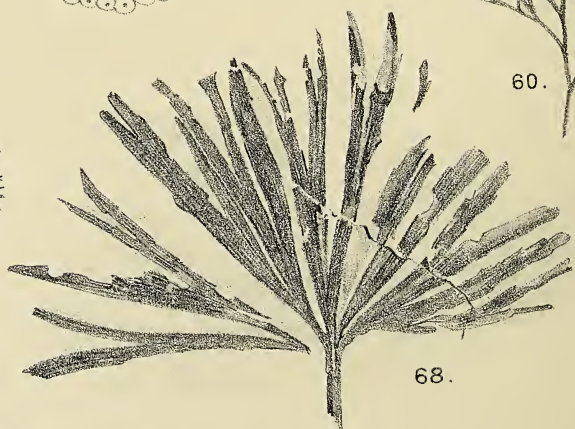
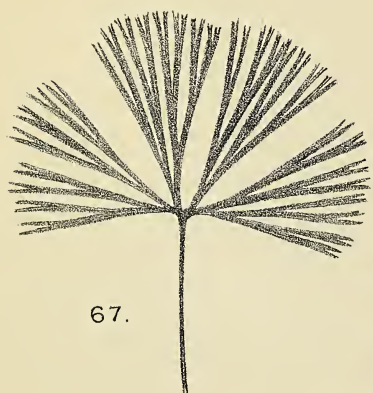
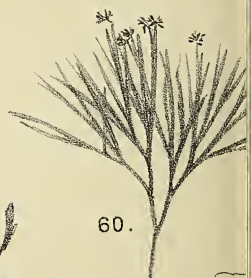
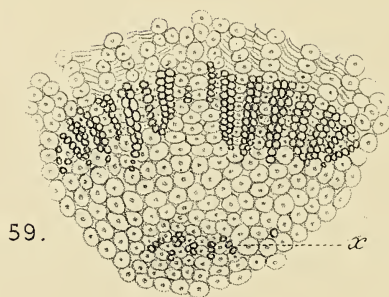
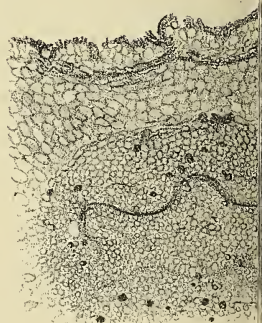
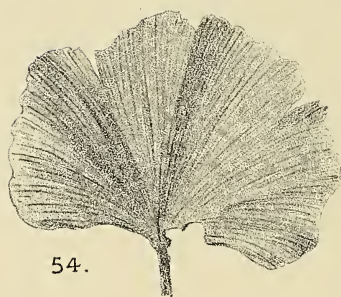
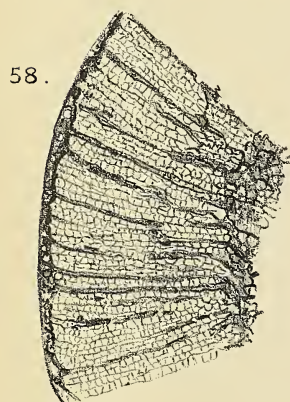
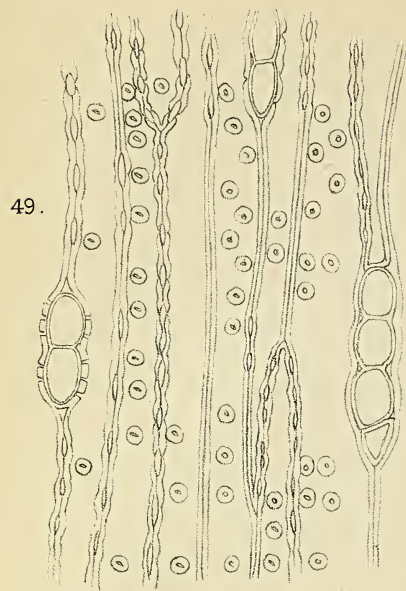
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SEWARD & GOWAN. — ON GINKGO.









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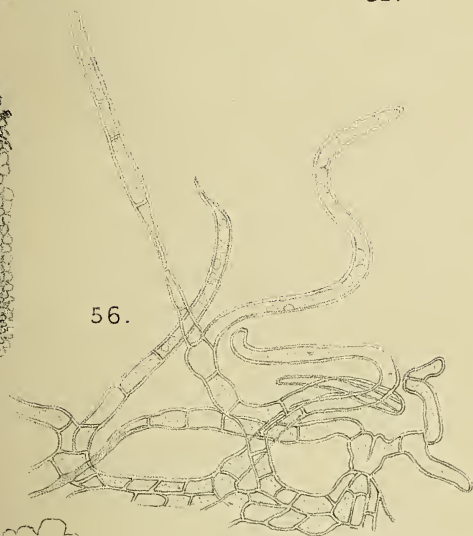
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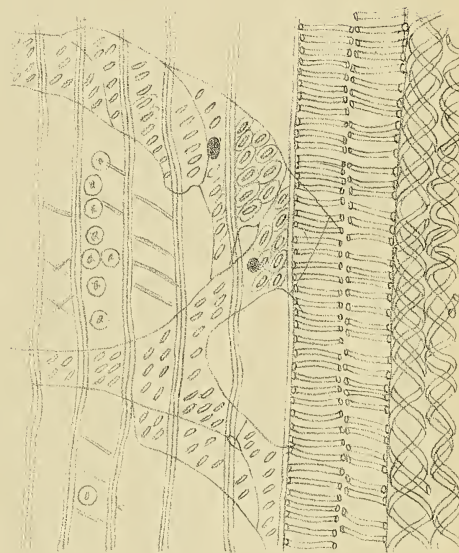


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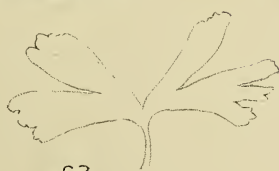


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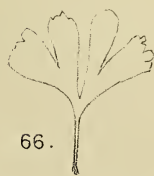
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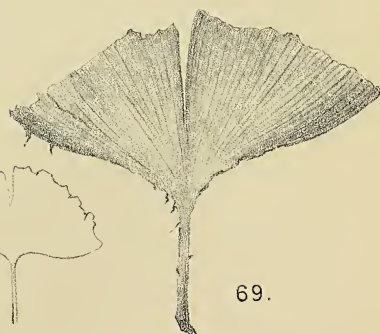
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J. Gowan del.

SEWARD — ON GINKGO.  
& GOWAN.





## NOTES.

**ON THE RESERVE-CARBOHYDRATES OF THE BULB OF THE HYACINTH** (*Hyacinthus orientalis*, L.).—Leclerc du Sablon states, contrary to previous workers on the subject, that the reserve-carbohydrate associated with starch in the bulb-scales of the Hyacinth is dextrin. In a paper in 1898<sup>1</sup>, on the carbohydrate reserve-materials of bulbs and tubers, he characterizes the Hyacinth as agreeing with *Ophrys*, *Lilium*, and *Tulipa*, in possessing both starch and dextrin in its organs for storage. In a more recent paper<sup>2</sup>, he again draws attention to the occurrence of dextrin, as a reserve material, in the Hyacinth, Tulip, Lily, and Asphodel.

Chevastelon<sup>3</sup> in 1894 examined amongst other reserve-organs the Hyacinth bulb, and describes the soluble reserve-carbohydrate as a kind of inulin.

In a recent publication<sup>4</sup> on the carbohydrates of Monocotyledons, I have called attention to Chevastelon's work, and also have placed the Hyacinth bulb, from my own microscopic and microchemical examinations, in the group of reserve-organs containing both starch and an inulin easily soluble in cold water. Since the presence of inulin in this bulb was inferred, and not proved chemically, I have deemed it expedient to perform the experiment now to be described.

The scales of the bulb were mashed up and extracted with cold water. Alcohol was gradually added to the extract. Some flocculent matter first separates out; this is chiefly the mucilage of the raphide-cells, which is rendered insoluble by a lower percentage of alcohol

<sup>1</sup> Leclerc du Sablon, Les réserves des bulbes et des tubercules. (Revue Générale de Botanique, 1898, p. 387.)

<sup>2</sup> Ibid., Sur la dextrine considérée comme matière de réserve. (Comptes Rendus, cxxviii, 1899, pp. 944, 945.)

<sup>3</sup> Chevastelon, J., Pharmacie, 1895 [6], pp. 83-6, from Inaug. Diss. Paris, 1894.

<sup>4</sup> Parkin, Phil. Trans. Royal Soc. Lond., Series B, vol. cxc (1899), pp. 56 and 72.

than the inulin. The mucilage is removed by filtering and more alcohol is added, when a dense white precipitate is produced, which gradually settles to the bottom of the vessel. This is the inulin. The alcohol is poured off and the precipitate dissolved in a little cold water, and the solution treated again in a similar manner with alcohol. The operation can be repeated a third time, when a fairly pure substance is obtained.

1.457 grams of this, dried at  $100^{\circ}$  till constant in weight, was taken and dissolved in water, and the volume made up to 100 cubic centimetres.

Angle of rotation in the 200 mm.  
tube of the polarimeter =  $-1.2^{\circ}$ .

50 c.c. were acidified with oxalic acid to the extent of 1 per cent. and heated for an hour on a water bath.

After hydrolysis the angle of  
rotation in the 200 mm. tube =  $-2.5^{\circ}$ .

I then took 10 c.c. of the hydrolysed portion, and boiled this for a definite time with a definite amount of Fehling's cupric reagent. The cuprous oxide was collected on an asbestos plug, reduced to copper in a current of hydrogen and weighed.

10 c.c. yielded 0.2475 gram of copper. This calculates out to 1.363 gram of fructose (levulose) in the 100 c.c. Theoretically there should be 1.618 gram of fructose yielded by the 1.457 gram of inulin originally taken. The discrepancy is probably due to impurity in the inulin, or possibly to reversion-products formed in the hydrolysis.

The amount of fructose calculated from the opticity (taking the specific rotatory power of fructose at a temperature of  $15^{\circ}$  to be  $-98.8^{\circ}$ ) is 1.265 gram, which corresponds fairly well with that derived from the cupric-reducing power.

The specific rotation of the inulin, deduced from the observed angle, is about  $-45^{\circ}$ , a number agreeing closely with such obtained for other monocotyledonous inulins<sup>1</sup>.

The above results lead to the view that the carbohydrate in question is of the nature of inulin, yielding fructose on hydrolysis.

There seems then no doubt that the reserve-carbohydrate accompanying the starch in the Hyacinth bulb, is inulin rather than dextrin, using the word inulin in its extended sense for all those

<sup>1</sup> Tollens' Handbuch der Kohlenhydrate, Band ii, pp. 239, 240.



carbohydrates (polysaccharides) which are levorotatory, and which, on treatment with an acid, hydrolyse to fructose solely or at any rate in the main.

Leclerc du Sablon does not mention submitting the solution of the carbohydrate he extracted from the bulb to the polarimeter, which is the simplest and most satisfactory method of distinguishing between dextrin and inulin.

As far as investigations have gone, the inulins of plants can be arranged in three classes:

- (1) That found in the Compositae and allied orders, which is precipitated in the tissues by alcohol in the form of the well-known spherocrystals, and which is practically insoluble in cold water, requiring a temperature of 50° to 55° for its solution.
- (2) That characteristic of many Monocotyledons, e. g. *Scilla*, *Yucca*, *Phleum*, and the plant now before us; it is precipitated in an amorphous form, in the tissues, as a thick lining to the inside of the cell-wall and is readily soluble in cold water.
- (3) That found in the bulb-scales of species of *Galanthus* and *Leucojum*, which is precipitated in the tissues in an amorphous form, and which requires a temperature as high as 80° for its solution<sup>1</sup>.

On the other reserve-organs mentioned by Leclerc du Sablon as containing dextrin, I have not much to say. Only one of them, *Asphodel*, have I asserted to contain inulin, as does likewise *Chevas-telon*, and in all probability it does store an inulin similar in kind to that of the *Hyacinth*. In my paper the bulbs of *Lilium* and *Tulipa* are mentioned, among others, as not responding to the inulin tests, and so it is quite possible that the carbohydrate obtained from these by Leclerc du Sablon is dextrin in nature.

J. PARKIN.

CAMBRIDGE BOTANICAL LABORATORY,  
December, 1899.

**FORMATION OF AN IRREGULAR ENDODERMIS IN THE ROOTS OF RUSCUS sp.**—The root in which this irregular structure occurred was quite young, only a few xylem-elements in each group being lignified. As the sketch shows, there were fourteen

<sup>1</sup> Fischer, H., Cohn's Beiträge zur Biologie der Pflanzen, 1898, Bd. viii, Heft 1, pp. 89, 101, 102. Ehrhardt, Abstract Bot. Centralb., 1894, Bd. lx, p. 207. Parkin, loc. cit., pp. 59 and 64.

protoxylem- and phloem-groups. The vascular cylinder was entirely enclosed by the endodermis in the normal way, but in one place a cell of the endodermis was joined by its uncuticularised tangential wall to a complete ring of endodermis external to the central cylinder. No

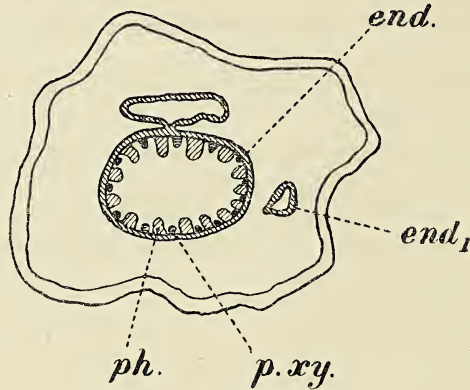


Fig. 8.

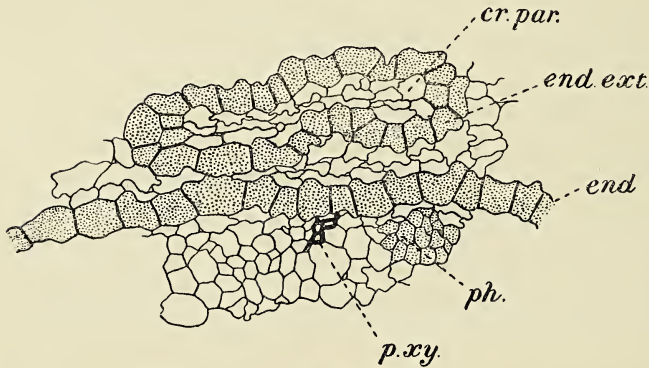


Fig. 9.

Fig. 8. Diagram illustrating the general arrangements of tissues in the transverse section of the root of *Ruscus* sp. Fig. 9. Part of the above more magnified to show the endodermis.

*End.*, endodermis; *end. ext.*, isolated ring of (abnormal) endodermis; *cr. par.*, crushed parenchyma; *ph.*, phloem; *p. xy.*, protoxylem.

vascular tissue of any kind was present within this ring, the elements consisting entirely of crushed parenchyma.

Further out in the cortex and on the opposite side of the root, a small isolated ring of endodermis was present, and this also enclosed

crushed parenchyma. This structure continued unchanged for a distance of more than an inch down the root, and there was no sign within that distance of the isolated ring fusing with the ring of endodermis enclosing the vascular cylinder. The root in which this structure occurred was abruptly twisted and bent, and this fact may possibly be in some way correlated with the appearance of the endodermis.

The chief points of interest to be noted in this anomalous structure are :—

First, the occurrence of such an anomaly in roots, which generally show great uniformity in their structure.

And secondly, the absence of all vascular tissue inside the abnormal endodermis, just as Pfitzer found to be the case occasionally in *Equisetum hyemale*<sup>1</sup>.

In view of the great stress which has been laid by Van Tieghem and others, in connexion with the stelar theory, on the endodermis as a layer of tissue of great morphological value, the first point is of especial interest, for the structure which is of critical importance in the stelar theory is thus proved to be capable of considerable variation even in the same plant.

The second point is of interest as illustrating an additional instance of the possible existence of a complete independence between the endodermis and all vascular tissue; i.e. the endodermis can be fully developed without any reference to the vascular tissue with which it is so generally associated.

F. J. LEWIS.

ROYAL COLLEGE OF SCIENCE, LONDON.

**THE ANATOMICAL STRUCTURE OF BOWENIA SPEC-TABILIS**, Hook.—Having through the kindness of the authorities of the Royal Gardens, Kew, obtained some seedlings of *Bowenia*, and having proceeded to examine their anatomical structure, I discovered the following characters in the vegetative axis.

In the extremely short young *stem* the central cylinder contains about three stout bundles consisting of a great deal of secondary tissue, and a large amount of parenchyma. Leaf-trace bundles occur

<sup>1</sup> Pfitzer, Ueber die Schutzscheide der deutschen Equisetaceen, Pringsh. Jahrb. f. wiss. Bot. vi, 1867, p. 320.



in the cortex. Lower down, just *beyond* the transitional region between stem and root, and where root-structure already prevails, the central stele is of considerable width, and the constituent bundles farther apart, owing to the expansion of the parenchymatous tissues. The large bundles composing the secondary tissues of the stele are now in places connected by delicate, few-layered bands of secondary vascular tissue, whose course is rather difficult to follow.

So far the structure is normal enough. But outside the central normal stele are seen, in transverse section, other strands which, there can be no doubt, represent the extrafascicular vascular tissue of *Cycas*, *Macrozamia*, and *Encephalartos*. Now this extrafascicular vascular tissue, which, at this youthful stage, only occurs outside a small part of the circumference of the central stele, has this peculiarity: that it consists of two distinct parts, viz. an outermost normally orientated and an *innermost band whose parts are abnormally, i. e. inversely orientated*, with the xylem directed outwards and the phloem inwards. Of these two well-marked portions of the extrafascicular tissue, the inner—or that with inverted orientation—is, at this stage of the plant's life-history, the best developed of the two. Both portions, in accordance with the youthful age of the plant, are as yet in a very rudimentary stage of development, the xylem and phloem being in fact but just sufficiently advanced in differentiation for the determination of the above salient characters.

The above structure I regard as homologous with, although a slight modification of, that described by Gregg in the root of *Cycas Seemannii*, Al. Br., and by myself in the root of *Cycas revoluta*, Thunb., and the stem of *Macrozamia Fraseri*, Miq.

It appears to me, moreover, to distinctly support the view put forward in the paper dealing with the latter plant, that the vascular tissues (at least, the extrafascicular zones) are derived by modification from those of plants like the Medulloseae, which normally possess vascular strands, each of which exhibits the mutually-inverted portions above described.

W. C. WORSDELL, Kew.

**A NEW CARDIOCARPON-BEARING STROBILUS.**—The writers have latterly each met with specimens of *Cardiocarpon*, as figured by the late Professor Williamson and other authors, but attached to a central axis in a strobiloid form (resembling a *Lepido-*

*strobilus*), which has not to their knowledge yet been found or described by any other persons.

The first strobilus was found in material from Moorside, near Oldham, some time ago. Latterly the writers have found portions of a strobilus, transverse and otherwise, and also a complete strobilus, in material collected by one of them from Hough Hill, Stalybridge. All the specimens are from the calcareous nodules found in the Upper Foot and Gannister Mines of the Lower Coal-Measures.

The interest attaching to these fossils lies in the fruits being borne in a spike-like cluster or strobiloid form, the larger and more matured fruits or carpels being at the base, and gradually decreasing upwards to the top of the cone. It would seem that this manner of growth would account for the difference in size of the fruit as found detached, and figured by authors.

The specimens have been placed in the hands of Dr. D. H. Scott, Hon. Keeper of the Jodrell Laboratory, Royal Gardens, Kew, for further investigation and description.

G. WILD, Bardsley, Ashton-under-Lyne.

J. LOMAX, Moses Gate, Bolton.

ERRATUM.

Annals of Botany, Vol. XIII.

In Professor Bertrand's note on the Structure of the Stem of a Ribbed Sigillaria, p. 607, for *Haidinghen* read *Hardinghen*, throughout.



# Nuclear and Cell Division in *Dictyota dichotoma*.

BY

DAVID M. MOTTIER,

*Professor of Botany in the Indiana University.*

—+—  
With Plate XI.  
—+—

AMONG the Brown Algae certain representatives, as *Fucus*, *Stypocaulon*, *Sphaceleria*, and *Dictyota*, have, in recent years, been found to afford favourable material for the investigations of cytologists dealing with nuclear and cell-division. One of these genera, *Stypocaulon* (Swingle, '97), was the first Alga in which the persistence of the centrosome was, without doubt, demonstrated throughout successive generations of vegetative cells. The discovery, in 1897, of motile antherozoids in *Dictyota* and *Taonia* by Williams ('97) has aroused a renewed interest in the *Dictyotaceae* from the standpoint of evolution, and my own observations will show, I think, that in the tetraspore mother-cells of this plant we have a rather favourable object for a study of the centrosphere and the development of the karyokinetic spindle, as well as the formation of the cell-plate.

A short sojourn at the Zoological Station in Naples, in the spring of 1898, enabled me to collect material and to make a preliminary study of the centrosphere, the results of which have already been published in the *Berichte der*

[*Annals of Botany*, Vol. XIV. No. LIV. June, 1900.]

Deutschen Botanischen Gesellschaft. Since that time I have been able to make a somewhat exhaustive study of the entire process of nuclear and cell-division in both vegetative cells and tetraspore mother-cells, and the results of this study are set forth in the following pages.

#### METHOD.

To the cytologist the method by which results are obtained is always of prime importance, since the improvement of older methods or the discovery of new ones is one of the principal channels through which progress in cytology is possible; and as certain features in the method used proved somewhat surprising, a brief statement of the same may be useful to others pursuing similar lines of investigation.

In the beginning of my study the same combination of chromic-osmic-acetic acid, which gave such remarkably good results for the pollen mother-cells in *Lilium* and other higher plants, was used, except that the solution of chromic acid was made from sea water, namely: Solution A,

1 or $\frac{1}{2}$ % chromic acid (in sea water)	16 cc.
2 % osmic acid	3 cc.
Glacial acetic acid	1 cc.

If the objects were left in the fluid for a longer time, one-half per cent. chromic acid was used instead of one per cent.

Material fixed in this fluid, when worked up immediately, proved to be so blackened, that preparations, even of very thin sections, were unsatisfactory and generally worthless. Much time and patience were required to determine the amount of osmic acid necessary to fix cytoplasmic structures well, with as little blackening as possible. At first the above solution A was diluted with an equal volume of sea water, which I shall designate solution B. When the solution was diluted one-half, one per cent. chromic acid was always used in the original. This also proved to blacken too much. Several solutions were then tried, in which one to four drops

(1 drop being about one-twentieth of a cc.) of the two per cent. osmic acid were added to ten cubic centimetres of chromic acid and about one-half cubic centimetre of glacial acetic acid. One drop of the two per cent. osmic acid proved sufficient in some cases to give good results. The formula would then be as follows :—

1 % chromic acid (in sea water)	10 cc.
2 % osmic acid 1 to 3 drops (1 drop = .05 cc.)	
Glacial acetic acid . . . . .	.5 cc.

It was upon material fixed in this last-named solution that the results of my preliminary paper were obtained.

Pieces of the plant, on being thrown into these fluids, became dark almost at once, but after a short time the dark colour of the thallus disappeared owing to the oxidation caused by the chromic acid.

Since it was impossible to make in any way an exhaustive study during my stay at the Station in Naples, material fixed in the above solutions, and also in various strengths of Vom Rath's platinum-chloride-osmic-acetic acid, was brought gradually into seventy per cent. alcohol, where it lay until ready for use, which was during the following December and January, 1899.

In the course of investigation, material which had been fixed in the solutions A and B previously mentioned, gave, to my agreeable surprise, excellent results, for many preparations made from such material left little to be desired. Vom Rath's fluid gave less satisfactory results.

In thin sections the blackening was readily removed by allowing the slide to remain for a short time in a bath of alcohol and hydrogen peroxide (70 cc. of 80 % alcohol and 30 cc. of hydrogen peroxide).

Material fixed in solutions weak in osmic acid gave good results also after lying in the seventy per cent. alcohol for the time mentioned, but differential staining was more difficult and less satisfactory. The radiations and spindle-fibres retain the stain with less avidity, consequently the finer



details of both nuclear figure and remaining cytoplasm are less sharply defined. The staining process was the same as that used by myself in cytological studies on the higher plants (Mottier, '97, '98).

#### THE CYTOPLASM.

The following remarks pertain to the tetraspore mother-cell, unless otherwise stated.

The cytoplasm, especially during the preparation for the process of nuclear division, reveals two well-defined and sharply differentiated portions, a fibrillar portion, the kinoplasm, which is always associated with the nucleus and which plays the most important rôle in the karyokinetic process, and the remaining alveolar portion or trophoplasm of Strasburger. The alveoli (*Waben* of German authors) are relatively large and generally uniform in size (Plate XI, Figs. 2, 8); their walls or lamellae are generally smooth and of a uniform thickness. Upon the lamellae and in the angles of their meshes are often present numerous small granules which impart the granular appearance to the alveolar structure. Among the larger alveoli there are also present, especially in the vicinity of the nucleus, smaller meshes of granular plasma. This is partly fibrillar and partly alveolar.

In neither the reproductive nor the vegetative cells of *Dictyota* do we have a differentiation of the alveolar plasma into two sharply defined zones as in *Stypocaulon* (Swingle, '97, Figs. 1 and 4), namely a region of rather uniform and small meshes surrounding the nucleus and one of much larger meshes occupying the remaining part of the cell.

Regarding the terms *kinoplasm* and *trophoplasm*, Strasburger ('98), in a critical and exhaustive discussion of the cell-wall and its origin, proposes for these physiological terms others of morphological significance, namely, filar plasma and alveolar plasma. He does not intend, it seems, that the latter terms should entirely replace the former, but that they be used in connexion with them (p. 517).



‘Da die von mir vorgeschlagenen Bezeichnungen inzwischen eine ziemliche Verbreitung fanden, so möchte ich sie nicht ganz fallen lassen. Auch scheinen sie mir bei unserer jetzigen Kenntniss vom Zellenleben immerhin noch brauchbar zu sein; andererseits kann ich jetzt auch morphologische Bezeichnungen vorschlagen, die sich vielleicht neben den physiologischen zum Gebrauch empfehlen würden.’

It may be reasonably questioned whether the morphological terms are more fitting than the physiological, for, as it will be shown in what follows, the kinoplasm may not necessarily exist in the form of fibrillae, or that condition in which it is found when in the active state during karyokinesis. In the opinion of the writer the term *kinoplasm* is an especially fortunate one, since it cannot be doubted for an instant that, during nuclear division, there exists in the cytoplasm a differentiation which is the expression of a division of labour, and that the kinoplasm is more directly concerned in the mechanics of nuclear division than the remaining cytoplasm. It is not necessary to assume that kinoplasm is a morphological constituent of the cytoplasm, yet there is much to support this view, as the investigations of Strasburger previously cited, and my own studies on pollen and embryosac mother-cells amply show. Even were there no such evidence, the term would be justified merely as one of description to designate that part of the cytoplasm which is instrumental in the formation of the nuclear spindle.

The term trophoplasm is probably less fortunate and may lead to misunderstanding. The term kinoplasm I shall use in the following pages in its original sense; the word trophoplasm will not be used at all. It is not necessary to assume that all the protoplasm of a cell is either alveolar or fibrillar to explain the phenomena presented in a well-fixed and stained cell. As plant-cells plainly show, both forms may be present in the same cell, the net-like threadwork merging almost imperceptibly into the alveolar structure, as is undoubtedly the case in *Dictyota*.

In the tetraspore mother-cells the chloroplasts are numerous,

though smaller than in the ordinary epidermal or superficial cells of the thallus. This is because of their rapid multiplication during the growth of the tetraspore mother-cells. In the medullary or central cells of the thallus only a few smaller chloroplasts are present. They may be elongated, elliptical, bean-shaped, or often globular. They do not as a rule lie in the cavities of the alveoli, but are applied to their walls or to the threads of the cytoplasmic framework. Even after the process of fixing, imbedding, and staining, they still retain their brown colour, which, however, is paler than in the living cell.

#### THE NUCLEUS.

As in *Stypocaulon*, there is only one nucleus in each cell. In one case an apical cell was observed in which two nuclei in the resting condition lay very close together. In this instance nuclear division had been completed before a cell-plate had put in an appearance. Whether this is purely an exceptional case, or whether in the apical cell, cell-division is later in point of time than in other vegetative cells could not be decided. It is highly probable that this is exceptional, for in other vegetative cells cell-division was well under way before the daughter nuclei had passed into the complete resting stage. When the tetraspore mother-cell has increased considerably in size, the nucleus also becomes proportionately enlarged. Indeed, as pointed out by Swingle ('97) for *Stypocaulon*, the size of the nucleus seems to be in some degree proportional to the size of the cell. The nucleus contains one very large nucleolus, invariably much vacuolated, and a fine linin-reticulum, upon which are distributed fine granules varying somewhat in size. Many of these granules, but not all, constitute the chromatin. With the exception of the larger ones, which are more densely stained, they present the same greyish colour as the linin.

The amount of chromatin in the primary nucleus of the tetraspore mother-cell is certainly less in proportion to the size of the nucleus than in the vegetative cells. In fact

there is much to indicate that this amount is nearly the same in all nuclei, those of vegetative cells appearing on account of their smaller size richer in this substance. In the primary nucleus, during the prophase, there is never developed the rather regular and uniform chromatin-spirem, such as is present in the vegetative cells (compare Figs. 2 and 18), but instead the chromatin is collected into masses more or less isolated from which are eventually formed the chromosomes (Fig. 2). In the vegetative cells, on the other hand, there is developed a chromatin-spirem, as in the higher plants (Fig. 18).

#### KARYOKINESIS IN THE TETRASPORE MOTHER-CELLS.

The development of the karyokinetic spindle in the Phaeophyceae has been studied recently by Strasburger ('97), and by Farmer and Williams ('98) in *Fucus*, and by Swingle ('97) in *Stypocaulon*.

In the vegetative cells of *Stypocaulon*, according to Swingle (l. c., p. 315), the first indication of karyokinesis is the division of a small deeply stained granule or granules, adhering closely to the nuclear membrane, from which kinoplasmic fibres radiate. This granule or pair of granules is probably homologous with the *centrosome* or *microcentrum* of Heidenhain, and possibly with the *centriole* of Boveri, and for the sake of convenience each body or pair of granules is spoken of as a centrosome. The division of the cluster of kinoplasmic radiations takes place simultaneously with the separation of the daughter centrosomes (l. c., Figs. 9 and 2, Taf. XV; Figs. 13 and 14, Taf. XVI). This all happens before the cell-plate which follows the division of the nucleus is formed. After the formation of the cell-plate the daughter centrosomes have assumed their definite positions. The angular distance on the surface of the nucleus traversed by the separating daughter centrosomes varies from  $180^{\circ}$  to  $135^{\circ}$  in the different successive segments. While this is taking place, the chromatin framework and the nucleolus undergo certain changes



which need not be mentioned here. During these processes also the dark bodies at the poles, which were previously dumb-bell shaped, have become thin and sharply bent at the middle (l. c., Fig. 7, upper pole, and Fig. 10, Taf. XV). They are concealed by the densely crowded kinoplasmic fibres which penetrate the membrane and enter the cavity of the nucleus.

These filaments, spreading out laterally, grow towards the centre of the nuclear cavity (l. c., Fig. 17, Taf. XVI). At about this stage the very small, deeply staining chromosomes begin to collect at the ends of the entering fibres (l. c., Fig. 18, Taf. XVI). Even before the chromosomes collect at the ends of the spindle-fibres, it may be seen that they end in a blunt point, or in a little granule or knob whose diameter is, however, only slightly larger than the fibre. As the spindle-fibres penetrate further inward, the chromosomes are heaped up in an irregular mass near the centre of the nuclear cavity (l. c., Fig. 20, Taf. XVI). The polar radiations are less pronounced and the nucleolus has entirely disappeared. The next stage is that of the equatorial plate, where the chromosomes are arranged in a plane, others forming the base of two pyramids of spindle-fibres (l. c., Fig. 21, Taf. XVI).

Strasburger's account of the formation of the spindle in *Fucus* is much less complete as to details, but there is no doubt that it is similar to that of *Stypocaulon*.

In describing the process in *Dictyota* I shall begin with the first nuclear division in the tetraspore mother-cell at the time when the two centrospheres are on exactly opposite sides of the nucleus.

As soon as the tetraspore mother-cell has increased sufficiently in size to be hemispherical in shape or even a little larger, and to appear very conspicuous beside the neighbouring superficial cells, as I have described in my preliminary note (Mottier, '98), there appear on opposite sides of the nucleus two large, sharply-defined clusters or asters of kinoplasmic fibres radiating from a rod-shaped body often slightly bent,



lying either closely applied to the nuclear membrane or at some little distance from it (Plate XI, Fig. 1). This rod-shaped body is the centrosome, which, together with its kinoplasmic radiations, I shall speak of as the centrosphere. The planes of the longitudinal axes of the centrosomes may be parallel or form various angles with each other. In Fig. 1 the centrosome at the upper side of the nucleus is seen from the side, the lower from the end. Viewed from the pole the centrosome is always rod-shaped. The kinoplasmic fibres radiate in all directions into the cytoplasm, where they pass over into the framework of the same (Fig. 2). On the side next the nucleus they may run parallel with its wall for some distance. Near the nucleus the cytoplasm is more granular, with smaller meshes. It is more nearly a thread-like network than alveolar in structure, and appears with differential staining as kinoplasm. This very fine granular threadwork often extends in among the radiations of the centrosphere. The chloroplasts may extend close up to the nucleus (Fig. 3) or remain some distance from it (Fig. 2).

As karyokinesis progresses, the centrospheres become more conspicuous, the number of kinoplasmic radiations undoubtedly increasing.

While this is going on certain changes have taken place in the nucleus. At first there is present a large vacuolated nucleolus and a fine linin-reticulum with rather large meshes, upon which are arranged small and nearly uniform granules, all of which, as previously mentioned, are not chromatin. The chromatin now begins to collect into larger and somewhat irregular masses that finally become the chromosomes. The nucleolus becomes more vacuolated and soon disappears. The nuclear cavity presents a more granular appearance, the granules staining more densely.

The kinoplasmic fibres now penetrate the membrane of the nucleus and enter its cavity, while at the same time the polar radiations diminish in number (Figs. 2 and 3). They do not enter the cavity so uniformly as in *Stypocaulon*, but some proceed much in advance of others. These fibres are

not truncated at the ends, but somewhat attenuated or rather of a uniform diameter throughout. On entering, some pass straight toward the centre of the nucleus, while others diverge toward the sides. As these fibres approach from opposite sides of the nucleus, they tend to collect the chromosomes into an irregular mass in the equatorial region, where they finally form the nuclear plate (Figs. 4, 5, 6). Certain of the fibres coming from opposite sides seem to unite at their ends to form the continuous spindle-fibres which extend from pole to pole; others fasten themselves to the chromosomes, and still others diverge toward the nuclear wall in the equatorial region. In the mature spindle, therefore, the fibres present the following orientation: those radiating from the poles, the continuous spindle-fibres extending uninterruptedly from pole to pole, those running from the poles to the chromosomes, and the fibres which diverge from the poles toward the equatorial region and end in the cytoplasm. Of the latter there are fewer in the mature spindle than at an earlier stage. In addition to the phenomena just described, several others may now be mentioned. Sometimes (Fig. 3) the nuclear membrane is apparently pushed in at the poles where the spindle-fibres penetrate. This may be due in part to a slight shrinkage. Up to this stage the nuclear membrane is unbroken. As the spindle-fibres enter the nucleus, its membrane begins to disappear at the poles, and very soon it is no longer to be recognized as such at those points, while at the sides it remains almost unchanged until a later stage or after the spindle is fully formed (Figs. 4, 5, 6), when all traces of nuclear membrane finally disappear. Thus the spindle, with the exception of the polar radiations, lies within the nuclear cavity, its fibres, however, being of cytoplasmic origin. How far any nuclear substance contributes to the formation of the spindle is difficult to decide.

As previously mentioned, the polar radiations become much less pronounced and apparently fewer in number as soon as the spindle-fibres enter the nucleus. The centrosomes also become smaller (Fig. 5), being frequently almost concealed

by the closely arranged kinoplasmic radiations, and the nucleolus rapidly disappears.

On the disappearance of the nucleolus numerous granules appear in the nucleus, which stain deeply, closely resembling the chromatin granules. In the mean time the chromosomes increase in size, and it seems reasonable to suppose that the nucleolar substance contributes materially to their growth. The behaviour of the nucleolus during the second mitosis, as will be shown later, seems to strengthen this view.

The chromosomes when arranged in the equatorial plate appear, especially when crowded together, which is often the case, as rounded lumps. A careful study in favourable cases shows clearly that each chromosome is either in the shape of a ring, so contracted as to leave scarcely any central space, such, for example, as is known to exist in higher plants (*Podophyllum* and *Helleborus*), when each segment or daughter chromosome forms one-half of the ring, or it may be in the form of a short thick U (Figs. 5 and 6). Sixteen chromosomes, the reduced number, are present in the first mitosis. To this part of the problem I shall return in a later paragraph.

While on the way to the poles the daughter chromosomes sometimes fuse with one another to form large masses; this is especially so in the second mitosis. In the construction of the daughter nuclei one or more larger masses of chromatin are formed by the chromosomes, a nucleolus appears near the chromatin mass or masses, and a nuclear membrane is laid down (Fig. 8). The membrane is unquestionably formed through the agency of the kinoplasmic fibres. The centrosome increases in size and the polar radiations are more distinct than in the spindle stage. The connecting fibres usually persist until the nuclear membrane is present, but a little later they disappear entirely. The chromatin mass gradually becoming less dense, soon disintegrates and the daughter nucleus passes into the resting condition (Figs. 9, 10).

During the reconstruction of the daughter nucleus the centrosome divides longitudinally, as it seems, the daughter



segments separating first at one end, so that a V-shaped figure immediately results (Fig. 9). The radiations are also divided, as a cluster is attached to each daughter centrosome. It is inferred that the centrosome splits longitudinally in division, from the manner in which the kinoplasmic radiations are attached to the daughter centrosomes, and from the fact that these bodies are always rod-shaped and nearly of the same length.

The daughter centrosomes now separate, moving along the nuclear membrane, but do not, as in the first mitosis, traverse an angular distance of  $180^\circ$  before the formation of the spindle begins (Figs. 10, 12, 13). From each centrosome a diverging bundle of fibres enters the nucleus, which contains a very fine network of linin with small scattered chromatin masses and a large nucleolus (Fig. 11). In this figure only one pole of the spindle-rudiment is shown, the other being in another section. At a little later stage these fibres form cone-shaped collections with their bases directed toward the chromatin, and a deeply staining mass, which is probably the disorganizing nucleolus (Fig. 12). This stage of the karyokinetic figure bears a striking resemblance to that observed by Harper ('97) in certain Ascomycetes. As the spindle-fibres within the nucleus increase in number, the polar radiations diminish, so that often only very feeble asters remain (Figs. 12, 13). The bases of the two cones of fibres unite to form a curved spindle, while at the same time the chromatin is arranged in the equatorial region; the nucleolar mass gradually becomes less and finally disappears (Fig. 13). The granular network still persists until a later stage, and seems to take no part in the formation of the spindle, or, at most, plays only a secondary rôle. The nuclear membrane disappears soon after the spindle is mature, generally before metakinesis, but the outline of the nuclear cavity now occupied by the karyokinetic figure may be distinguished at a later stage. The spindle of the second mitosis is smaller than that of the first, and may remain slightly curved until metakinesis. The orientation of its fibres is the same, and the

centrosomes undergo a similar diminution in size. The invariable tendency of the chromosomes to collect and fuse into larger masses has made it impossible to determine accurately their number in this division. In the equatorial plate often only two large chromatin masses are seen (Figs. 14 and 15), but from the number that can be clearly made out in the first division one cannot assume that these two masses represent only two chromosomes.

The condition of the daughter nuclei presents nothing different from what has already been described for the preceding mitosis. The connecting fibres likewise disappear entirely.

#### KARYOKINESIS IN VEGETATIVE CELLS.

In vegetative cells it is far more difficult and time-consuming to obtain as complete a series of stages in the development of the spindle as I was able to observe in the tetraspore mother-cells, but a sufficient number was found to indicate without much doubt what the process is, and to furnish data for a comparison with what has just been described.

The resting nucleus presents nothing out of the ordinary. Neither in the apical cell nor in any other was I able to demonstrate an aster or centrosphere at all times during the so-called resting period, but as soon as karyokinetic activity begins, an aster is always to be observed near the nucleus, even while the latter still presents the structure characteristic of the complete resting stage (Fig. 17). I have, however, observed the centrosomes with few but distinct radiations in the stalk-cell of a tetrasporangium, and often in other cells, which had been in the resting stage for a relatively long time, and which would probably not undergo further division.

From the very fine and characteristic linin-reticulum there is developed a distinct chromatin-spirem as in higher plants, a condition which does not obtain in tetraspore mother-cells (Fig. 18). The spindle reaches maturity before the membrane of the mother-nucleus disappears (Fig. 19). At this stage

and later no polar radiations were seen, or only a very few. The rod-shaped centrosomes were, however, always present (Figs. 19, 20, 21). In some cases observed, when the daughter nuclei were provided with membranes, the centrosomes possessed a few faint polar radiations (Fig. 22). The chromosomes are short, bent, slightly U-shaped, and about thirty-two in number. They lie closely crowded together, so that counting is difficult, and the number could not be exactly ascertained.

Regarding the body which is known as a centrosome among the Brown Algae, the old questions of morphological unity and phylogenetic origin still present themselves. It is not my purpose, however, to go into the voluminous literature touching upon these and similar questions, nor to discuss the various and conflicting theories that have been advanced from time to time. I shall content myself with a brief presentation of the facts as I have observed them in *Dictyota* and as they are known in *Fucus* and the *Sphacelariaceae*.

In the young tetraspore mother-cells of *Dictyota*, before they have increased noticeably in size, no centrosomes with their characteristic radiations were seen, nor were any observed in vegetative cells showing no sign of karyokinetic activity.

Throughout the two divisions in the tetraspore mother-cells the centrosome is unquestionably present at all stages, and in the germinating tetraspore and the first three or four cell-generations of the seedling plant resulting therefrom, the persistence of this body is undeniable. In Fig. 23 is shown a tetraspore which has begun to germinate. The structure of the nucleus itself does not indicate any karyokinetic activity, and we may say that it is in the resting condition, but the very evident centrospheres are in the position of the future poles of the spindle. The centrosomes are also rod-shaped, and, as elsewhere, can be seen with the greatest clearness. A daughter nucleus resulting from the first division in the spore is shown in Fig. 24. The same may be said of the next two or three successive generations



of cells. Further than this no observations were made in the seedling plant. A nucleus from an enlarging epidermal cell, previous to the division which will cut off the stalk-cell of the tetrasporangium, is seen in Fig. 25. Here, too, the centrospheres are well developed at the beginning of the prophase.

In the light of what we now know in *Fucus*, certain *Sphacelariaceae* and *Dictyota*, it seems evident that the body which in these plants has been called a centrosome, is one that persists from one cell-generation to another in vegetative and certain reproductive cells; it is capable of division, and is the centre of radiations that give rise to the karyokinetic spindle with its mantle and polar fibres. These facts seem to argue that the centrosome is a morphological unit of the cell, and by the cytologist who is eager to support this theory they may be deemed sufficient, but are they conclusive? While I admit that this evidence is the strongest that has, as yet, been advanced, nevertheless I am not convinced that it is conclusive proof, for there are other questions suggested here and in the higher plants which require further elucidation. We may appropriately inquire what the relation existing between the centrosome and its surrounding radiations is. Are the radiations outgrowths of the centrosome as a primary morphological unit or organ? Or are they constructed out of a differentiated part of the cytoplasm, say the kinoplasm, by the centrosome as a centre of activity? Or, finally, is the centrosome only a denser mass of kinoplasm formed by the meeting of the polar radiations and spindle-fibres?

The answer to these questions will necessarily influence our conception of the centrosome and also the significance of what we term kinoplasm.

When certain Algae and Fungi are considered alone, nothing is more plausible than that the radiations grow out of the centrosome as a primary source. In the higher plants, however, where there are no centrosomes, this argument will not stand, for there is no good reason to believe that the spindle-fibres in the Lily are not homologous with those in the Brown Algae or in Fungi. If kinoplasmic fibres which

go to make up the spindle are controlled in certain lower plants by the centrosomes as centres of force, how is it that the same fibres in higher plants construct a karyokinetic figure without centrosomes or individualized centres of force?

Harper ('99, p. 510), in the latest of his most excellent papers upon certain Fungi, is inclined to the view that the central body in the Ascomycetes is due to the meeting of spindle and ray-fibres. Speaking of the spindle in the ascus of *Lachnea scutellata*, he says: 'The ends of all these spindles in the equatorial plate stage are decidedly broad and blunt, and the central body in which they end is flat and disk-shaped, as in *Peziza Stevensoniana* and *Ascobolus*. It stains more densely than the rays or spindle-fibres, but there is no indication that it is more than a denser mass of kinoplasm formed by the meeting of the spindle and ray-fibres.'

Now there may be a question whether the 'central body' in the Ascomycetes is homologous with the centrosome in *Dictyota* or the other *Phaeophyceae*, but I am inclined to think that the behaviour of these bodies leaves little doubt as to their homology.

In *Dictyota* the centrosome is certainly not formed by the meeting of polar radiations and spindle-fibres, for it exists and divides when only one set of these fibres is present, and perhaps also when both sets are absent, as, for example, in the early prophase or resting stage of the primary nucleus of the tetraspore mother-cell.

With all the facts taken into consideration it seems to me that it is the kinoplasm which should hold the rank of morphological unit, and the centrosome be considered as an individualized part of the same, existing in that form in some organisms and not in others, and being the expression of certain activities of the living substance, which are still largely beyond the power of the investigator to comprehend and explain. It is the opinion of the writer that this view is more in harmony with all the facts now known in both plants and animals. It is also clear to me, moreover, that neither

this nor any other doctrine hitherto proposed is without objection. Any theory dealing with what we call a centrosome, wherever that may be found, must deal also with the kinoplasmic radiations and spindle-fibres associated with it, and any theory assuming the morphological unity of the centrosome similar to that of the nucleus, without at the same time taking into account the kinoplasmic fibres of the karyokinetic figure in higher plants, is inadequate.

#### THE FORMATION OF THE CELL-PLATE.

Within the last few years our knowledge of cell-formation has been greatly extended, and in some respects revolutionized, especially as regards the so-called processes of free cell-formation. The method by which the cell-plate or plasma-membranes are laid down in higher plants no longer finds such universal application as was formerly attributed to it.

The investigation of Strasburger ('97) and Swingle ('97) on the Phaeophyceae, and those of Harper ('97, '99) on the Ascomycetes and Phycomycetes, have opened up a new field of research.

The type of cell-plate formation in the Brown Algae differs from those described by Harper in the Fungi as well as from that in the higher plants. It seems possible, however, that in all of these apparently diverse types the plasma-membranes are laid down through the agency of the kinoplasm, although this may not be demonstrable as sharply differentiated radiations or as connecting fibres.

In *Fucus*, according to Strasburger ('97, p. 358), when the eight nuclei are distributed in the cytoplasm of the oögonium and the formation of the cell-plate begins, the centrosomes are no longer to be recognized and the kinoplasm cannot be distinguished from trophoplasm. The cell-plate arises in the trophoplasm, whose alveolar walls so arrange themselves as to form a continuous plasma-membrane.

‘Die Verbindungsfäden zwischen den Tochterkernen werden



hier ebenso wenig wie in den früher von mir studirten Sphacelariaceen ('92) vermehrt und bei der Bildung der Scheidewände verwerthet. Vielmehr entstehen die Scheidewände in dem wabigen Trophoplasma, dessen Waben sich entsprechend anordnen, um aus ihren Wänden fortlaufende Plasmaschichten herzustellen. Dass die sich bildenden Hautschichten, welche die Eier von einander trennen sollen, aus Trophoplasma hervorgehen, wird durch diese ihre Entstehung in den Wabenwänden des Trophoplasma noch nicht erwiesen. Ja, manche Erscheinung sprach hier dafür, dass dem nicht so sei. Ich beobachtete nämlich wiederholt in den Plasmalamellen, welche den neuen Hautschichten den Ursprung geben sollten, kleine, dicht aneinander schliessende Körner, die bei günstiger Anwendung des Orange-Verfahrens violett gefärbt erschienen (l. c., Fig. 22, Taf. XVII). Dort, wo die Hautschicht bereits erzeugt und eine Spaltung derselben erfolgt war, zeigten sich diese einfachen Körnchenplatten in zwei Platten entsprechend kleinerer Körnchen getrennt' (l. c., Fig. 22, Taf. XVII).

From the foregoing quotation it is evident that Strasburger attributes the actual formation of the plasma-membrane (*Hautschicht*), which constitutes the cell-plate, to the union of closely arranged kinoplasmic granules that afterwards split to give rise to the plasma-membranes forming respectively the contiguous surfaces of each daughter cell.

These kinoplasmic granules are compared to the thickenings of the connecting fibres, which takes place in cell-plate formation in higher plants, and in this respect the influence of his older doctrine is noticeable.

In *Stypocaulon* Swingle ('97) finds that the first indication of a cell-plate is seen in a group of alveolae which show a tendency to arrange themselves across the cell in a transverse plane (l. c., Figs. 3, 6, Taf. XV). As soon as this orientation of alveolae becomes more marked, the transverse alveolar lamellae form a more continuous plane, which in section appears as a very fine line.

During these changes neither an increase in the number of

connecting fibres between the nuclei nor any perceptible change whatever in the arrangement of the kinoplasm was to be seen. Only a few fibres or lines of force, indicated by the arrangement of the alveolae of the frothy plasma, extend from the nucleus of the apical cell to the seat of the cell-plate formation, and still fewer from the lower nucleus to the same place (l. c., Fig. 5, XV). It is certain, he further remarks, that if these be real fibres, they must be extremely delicate and not numerous enough to lead one for a moment to suppose that the cell-plate is laid down by any such process as in the Metaphytes. And although the entire phenomenon is a process which does not depend immediately upon the activity of the kinoplasm, yet it is not impossible that it takes place under the control of the nucleus exerted through the kinoplasmic fibres which connected the latter with the former cell-plate.

The development of the cell-plate in *Dictyota* resembles that in *Stypocaulon*. There is absolutely no visible trace of kinoplasmic connecting fibres between the nuclei, and in the region of the cell-plate the cytoplasm seems undifferentiated.

The plasma-membranes or cell-plates which will separate the four spores are laid down nearly simultaneously. In the regions where they are to appear, the cytoplasm, as elsewhere, except near the nuclei, reveals the same visible structure of alveolae, or perhaps a mixture of alveolar and thread-like network. Rather large and very small meshes are intermingled. The smaller-meshed structure is apparently more granular than that with larger meshes.

The first visible trace of a cell-plate is manifested by the transverse walls of the alveolae becoming perceptibly thicker and arranging themselves in such a way as to appear as an uneven or somewhat zigzag line in section (Fig. 16). In this cell-plate rudiment or *Anlage*, the walls of both large and small meshes take part. At first certain of the alveolar walls are thinner than others, so that the cell-plate seems interrupted at those places, but eventually and gradually it attains a uniform thickness. Very soon the cell-plate or

plasma-membrane is a uniform plane which in section appears as a smooth line.

The cell-plate is not always laid down everywhere simultaneously, but sometimes it appears at first more marked at the periphery. This seems to depend upon the position of the nuclei.

It must be admitted that this process of cell-plate formation is very different from what takes place among the higher plants, where we have a plasma-membrane formed by the connecting fibres. If there be kinoplasm present, it does not exist in the form of visible fibres, such as connecting fibres, but what is really seen, seems, in part at least, to be a direct transformation of the walls of the alveolae into the cell-plate.

All of this does not prove, however, that kinoplasm is not present, or that it is not instrumental in forming the plasma-membrane, which, there are strong reasons to believe, is composed of this substance.

Shortly before the cell-plate is marked as a continuous and somewhat irregular line, as described, the region in which it will appear is sometimes to be distinguished by the presence of fewer chloroplasts and somewhat finer meshes of the cytoplasm. The region stains more nearly like the kinoplasm near the nuclei, and when the plasma-membranes are formed, they also retain the gentian violet with greater avidity than the cytoplasm in general, especially if the fixing fluid were stronger in osmic acid. I have found it to be almost invariably true, both in pollen mother-cells and vegetative tissue of the higher plants, as well as in *Dictyota*, that in material fixed in the solutions stronger in osmic acid, e.g. solution A above, the spindle-fibres, or kinoplasm, stained a much deeper blue than when solutions weaker in osmic acid were used; and it may be added that the secret in the use of the chrom-osmic-acetic solution is to determine the proper amount of osmic acid necessary to make possible the best differential staining. The conclusion, therefore, is not unwarranted that in *Dictyota* the cell-plates are formed by means of kinoplasm.



This point of view does not assume that everything which remains blue after careful differential staining with the triple stain used is kinoplasm, nor is it claimed that the colour of any part of the plasma, resulting from a certain staining method, indicates the chemical nature of the part so stained. The colour may or may not do so; decisive proof is still wanting.

In vegetative cells the formation of the cell-plates is likewise interesting. The connecting fibres between the daughter nuclei disappear as such (Fig. 22), and the plasma-membrane seems to be laid down as in the delimitation of the tetraspores. It was not possible to follow the process in as great detail as in the tetrasporangium, but in the case observed the cell-plate began at the periphery of the cell and proceeded toward the centre (Fig. 22). The process is not a cleavage, as described by Harper for Zygomycetes, nor is it similar to the *Spirogyra* or *Cladophora* type, but is the same as described above. Fig. 22 is not magnified sufficiently to show the finer details clearly.

We have now to consider more fully the nature of the cell-plate, what its finer structure is, and whether it is formed double or as a single membrane, which afterwards splits to furnish the daughter cells with their respective portions of plasma-membrane.

In the pollen mother-cells of *Lilium* (l. c., '97) I showed that the cell-plate was formed through the direct agency of the connecting fibres, but the precise manner in which its substance was deposited was left an open question. 'Es unterliegt (l. c., p. 192), wie es mir scheint, keinem Zweifel, dass die Verbindungsfäden in directer Beziehung zu der Zellplattenbildung stehen, doch in welcher Weise, bleibe dahingestellt.' There the cell-plate is, at first, so far as can be seen, a single homogeneous disk separating the daughter protoplasts. Later, it appears double, when the contiguous surfaces of the two cells are provided each with a plasma-membrane (*Hautschicht*), between which a cell-wall is formed. Whether the cell-plate in either *Lilium* or *Dictyota* is laid

down as a single membrane, which afterwards splits, or whether two membranes are formed from the beginning, is difficult to decide. I am inclined to think that it is laid down as a double plasma-membrane, since evidence of the process of actual splitting was not seen, and immediately the cell-plate is formed, plasmolysis shows that each protoplast has its own plasma-membrane.

Concerning the form in which the cell-plate substance is deposited, there may be much diversity of opinion. In the higher plants, we may contend with Strasburger ('98, p. 514), that the plasma-membrane is formed by the lateral fusion of the thickened connecting fibres, when it would consist of many very small kinoplasmic rods; or we may assume that the cell-plate substance is deposited as a homogeneous fluid. Both in the higher plants and in *Dictyota* there is much that supports the second of these assumptions.

That the cell-plate in the higher plants is formed by the lateral union or fusion of the thickened connecting fibres may be seriously questioned, for in some cases these fibres do not thicken very appreciably in the equatorial region, nor do they lie sufficiently close to one another to enable the slightly thickened middle parts to meet and fuse laterally. I refer, for example, to the formation of the egg-cell in the embryo-sac of *Lilium Martagon* (Mottier, '98, Taf. III, Fig. 21). There the connecting fibres are only very slightly thickened in the region of the cell-plate, and they are too far apart to lead one to believe that the plasma-membrane is the result of the lateral fusion of the slight equatorial thickenings of these fibres. In this and in similar cases among higher plants, the conclusion seems justifiable that the cell-plate is formed by homogeneous plasma which is conveyed to the cell-plate region and deposited there by the connecting fibres.

In *Dictyota* there are no connecting fibres, neither does the cell-plate consist, at first, of a row of granules which split later to form the two plasma-membranes as described by Strasburger ('97, p. 359) for *Fucus*. The cell-plate does

contain very small granules similar to those observed everywhere in the framework of the cytoplasm, but in *Dictyota* they are not arranged in a definite plane to form a cell-plate. Their part in the process is secondary.

If, therefore, we accept the doctrine of a morphological differentiation of the cytoplasm, we have in *Dictyota* a cell-plate formed of kinoplasm, which neither exists in the form of connecting fibres, nor is its substance conveyed to the cell-plate region by such fibres. The possibility is not excluded, however, that the kinoplasm may exist in the form of fibrillae as claimed by Strasburger, and these fibrillae may constitute a fine network, distributed among the alveolae or applied to the walls or lamellae of the latter, when its fibrillar nature would be obscured; or it may exist in the form of a homogeneous fluid, evenly distributed over the firmer framework of the remaining cytoplasm, whether the latter be a thread-work or alveolar in structure.

It is the opinion of the writer, therefore, that, in such cases as *Dictyota* and *Stypocaulon*, the cell-plate or plasma-membrane is laid down by kinoplasm which is not differentiated in the form of conspicuous fibres, but that it exists in one of the forms just mentioned. There is reason to believe, also, that in many plants where a visible differentiation of the cytoplasm is not manifest, plasma-membranes are formed in a similar manner.

#### THE CHROMOSOMES.

As regards the question of chromosome-reduction, I am able to contribute comparatively little that is definite or conclusive.

The researches of Farmer and Williams and Strasburger have shown that in *Fucus* the reduced number of chromosomes appears in the oögonium, while in vegetative cells of the thallus twice that number is present. Strasburger ('97) finds that in the first nuclear division in the oögonium the reduced number appears, fourteen to sixteen having been



counted, and this number persists throughout the two succeeding mitoses. In vegetative cells the number is not far from thirty.

In *Dictyota* the first nuclear division in the tetraspore mother-cell, as already stated, reveals the reduced number. Here sixteen chromosomes were counted. In vegetative cells the smaller size and the crowding together of the chromosomes make counting more difficult and less accurate, but in several instances the number was found to be not less than thirty, and one may reasonably suppose that thirty-two is the normal number.

The two nuclear divisions in the tetraspore mother-cells are strikingly similar to those in spore mother-cells of higher plants. The tetraspore, being borne, as it seems, by the gametophyte, may not be homologous with the spores of higher plants, and consequently to speak of the nuclear divisions in the former as homologous with those in the latter might lead to confusion. The fact that these types of karyokinesis occur so universally in reproductive cells of widely divergent groups of organisms in both the vegetable and animal kingdoms forces upon one the conclusion that they are of the utmost theoretical importance.

If the reduction in the number of chromosomes should prove to be of as far reaching significance as is generally supposed, *Dictyota* offers, beyond any doubt, a most interesting field for speculation and further research.

Whether in the nuclei of the seedling plants arising from tetraspores the reduced number of chromosomes persists, and whether in the egg-cell the reduced number obtains, are problems which further research must solve. Unfortunately I was unable to obtain the proper stages of karyokinesis to ascertain the number of chromosomes in the cells of the seedling plants in question, nor was the division by which the stalk-cell of the oogonium was cut off, observed so that the number in the egg might be determined. In this respect it is of prime importance that the sequence of generations, arising from the tetraspores and from the fecundated egg, be ascertained with accuracy, and that the number

of chromosomes be known also in the vegetative cells of these generations.

For the solution of these problems a thorough and prolonged study at the sea-shore is necessary.

#### SUMMARY.

When we consider the facts presented in the foregoing paragraphs concerning the development of the karyokinetic figure, it will be seen that in *Dictyota* the nuclear spindle originates in two systems of kinoplasmic radiations or asters that lie close to the nuclear membrane and some distance from each other, generally on opposite sides of the nucleus. The radiations of each system are centered upon a very distinct rod-shaped body, the centrosome. For the sake of convenience I have spoken of the centrosome together with its radiations as a centrosphere. The centrosome is present during the two nuclear divisions which take place in the tetraspore mother-cell; it is also found in the germinating tetraspores, and its persistence has been observed in the first three or four cell-generations of the resulting seedling plant. In all vegetative cells of the thallus bearing tetraspores, the centrosome is readily recognized during karyokinetic activity. In the beginning of mitosis it divides longitudinally, and the daughter centrosomes with their radiations, or we may say the daughter centrospheres, move apart along the nuclear membrane to positions occupied by the poles of the future spindle. During the process of karyokinesis, the centrosomes may undergo a change in size, appearing smaller in the equatorial plate stage.

The fact of the persistence of the centrosome from one cell-generation to another, and of its multiplication by division, is certainly strong evidence in favour of its morphological unity as an organ of the cell. In the light of what is known in the higher plants, and owing to our limited knowledge of karyokinetic activity in the lower plants, I do not think we

are justified in attributing to the centrosome the morphological rank of an organ, such as the nucleus. It is, it seems to me, more in harmony with all facts pertaining to the subject to regard the centrosome as a special individualized part of the kinoplasm, existing in that form as the expression of certain activities which are as yet not well understood.

In comparing the development of the spindle in *Dictyota* with that of *Stypocaulon*, a close resemblance of the two is at once apparent. From each pole or centrosome kinoplasmic fibres enter the nuclear cavity. The membrane of the nucleus is unbroken at first, but later it gradually disappears at the poles. The remaining part of the membrane persists until a very late stage, sometimes after the spindle is mature.

The entering cones of spindle-fibres present less regular and even bases than those in *Stypocaulon*, a fact due to the more rapid growth of certain fibres. These cones meet in the equatorial region to form that part of the spindle between the poles. By this process the chromosomes are brought into the equatorial plate.

During the prophase of both divisions in the tetraspore mother-cell the behaviour of the chromatin differs strikingly from that in the higher plants. There is not developed here a regular and continuous chromatin-spirem which segments into the chromosomes, but these arise as isolated masses, often differing much in size. It gives the impression that the quantity of chromatin is not sufficient to form a continuous spirem. In the vegetative cells, on the contrary, there is developed a typical spirem as in the higher plants.

The reduced number of chromosomes, namely sixteen, appears in the first nuclear division in the tetraspore mother-cell. This division is quite analogous to the heterotypic division in spore mother-cells of most cormophytes. In the vegetative cells of the thallus bearing tetraspores the number is estimated at thirty-two.

The behaviour of the nucleolus, during both the development of the nuclear figure and the construction of the daughter



nuclei, indicates that this body represents a substance which is utilized by the chromatin and not by the spindle-fibres.

The development of the cell-plate or plasma-membrane in *Dictyota* belongs to the same type as that of *Stypocaulon*. No differentiated connecting fibres of any sort can be recognized. It seems that the apparently undifferentiated framework of the cytoplasm, consisting of large and small meshes in the immediate region of the cell-plate, is converted into a plasma-membrane. The behaviour of the cell-plate toward certain stains, and the character and behaviour of the cytoplasm in that region, immediately preceding the appearance of the plasma-membrane, as described in a foregoing paragraph, strongly suggest that the latter is not an actual transformation of the alveolar walls, but that the substance of the cell-plate is deposited by kinoplasm, which is present in the framework of the cytoplasm. The form in which this kinoplasm occurs here is difficult to determine. It does not matter, however, for our explanation, whether it takes on the form of a fibrous network or of alveolae.

As has already been mentioned, this type of cell-plate formation is different from that in the higher plants where connecting fibres are present, yet it bears a closer resemblance to the latter, perhaps, than to any of the several other methods known in the vegetable kingdom. It is not a cleavage as described by Harper ('99) for the *Phycomycetes*, nor is it in any way similar to the process of division by constriction that exists in either *Cladophora* or *Spirogyra*. Further investigation, especially in thallophytes and arche-goniaties, will probably bring to light transitions between the several distinct types of cell-formation that are now known among Algae, Fungi, and higher plants.

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## EXPLANATION OF FIGURES IN PLATE XI.

Illustrating Professor Mottier's Paper on Nuclear and Cell Division in *Dictyota*.

All figures were drawn from sections with the aid of the Abbé camera lucida and with the Zeiss apochromatic homogeneous immersion 2.0 mm., Apert. 1.40. Figs. 1-15, 24 and 25 with compensating ocular 6, image distance 30 cm. (i.e. distance from camera mirror to drawing paper). ( $\times 1200$ ). Figs. 9, 16-21, with compensating ocular 12, image distance 20 cm. ( $\times 1800$ ). Figs. 22 and 23, ocular 4, image distance 20 cm. ( $\times 625$ ).

Figs. 1-8 pertain to the first nuclear division in the tetraspore mother-cell.

Fig. 1. Nucleus in prophase with centrospheres on opposite sides; at the upper end the centrosome is seen from the side, that at the lower is seen from the end.

Fig. 2. A somewhat later stage. Nucleus with surrounding cytoplasm and chloroplasts. The centrospheres are large, with dense radiations; the chromatin has collected in larger irregular masses; the nucleolus is very large and vacuolated. To the left within the nucleus are numerous fine granules.

Fig. 3. The spindle-fibres have begun to penetrate the membrane and enter the nuclear cavity at the poles; chromatin masses large; nucleolus more finely vacuolate. At the poles the nuclear membrane is infolded, due probably to a slight shrinkage.

Fig. 4. The cones of penetrating fibres are much larger; a few of the fibres have met at the equator to form the continuous spindle-fibres extending from pole to pole. The chromosomes are collecting in the region of the nuclear plate. The nucleolus has almost or quite disappeared, while numerous smaller but densely staining granules are present; a fine threadwork, presumably linin, is also to be seen.

Fig. 5. A nearly mature spindle. The centrosomes are smaller and partly obscured by the numerous fine radiations and spindle-fibres centred upon them; at the sides the nuclear membrane is still preserved.

Fig. 6. A mature spindle, showing chromosomes arranged regularly in equatorial plate, and the following orientation of spindle-fibres: those extending uninterruptedly from pole to pole; the bundles of contracting fibres attached to the chromosomes and extending to the centrosomes; the mantle-fibres, or those diverging toward the nuclear membrane in the direction of the equator and the polar radiations. As in the two preceding figures traces of the nuclear membrane are visible at the sides.

Fig. 7. One end of a karyokinetic figure in the anaphase. The chromatin masses never approach nearer to the centrosome.

Fig. 8. Two daughter nuclei with surrounding cytoplasm; the nuclear membranes have just been laid down; the chromatin is in the form of a lumpy mass, near which is a very evident nucleolus, and a linin network with granules is also present. The connecting fibres have nearly disappeared, while the polar radiations have become more pronounced and the centrosomes larger in size.

Figs. 9-15. Division of the daughter nucleus.

Fig. 9. Daughter nucleus at a little later stage than Fig. 8, more highly magnified. The centrosome has just divided, the segments having separated at one end, but still almost touching at the other, so as to form a shallow V-shaped figure. The attachment of the fibres to the daughter segments suggests that the former exert a pulling force upon the latter.

Fig. 10. A later stage in which the centrospheres have moved apart a short distance. The nucleus reveals the structure of the resting condition.

Fig. 11. The beginning of the formation of the spindle; only one pole is shown, the other lying in the next section. As in the corresponding stage of the preceding division, the fibres of the spindle-cones are of unequal length, and the nuclear membrane is as yet unbroken. The nucleolus is vacuolate; the chromatin is represented by small but densely staining masses, and the linin is in the form of a small-meshed and finely granular threadwork.

Fig. 12. The centrospheres do not lie upon exactly opposite sides of the nucleus. (This figure lay somewhat obliquely to the plane of the section, so that a con-



siderable change of focus was necessary in drawing to include both centrospheres, and for this reason the centrosomes are a little nearer in the drawing than is really the case.) The spindle-cones, now well developed, have their bases turned toward the masses of chromatin and disorganized nucleolus; the polar radiations are few and indistinct.

Fig. 13. The cones of fibres have formed a curved spindle; the nuclear membrane is still unbroken, except perhaps directly at the centrosomes.

Fig. 14. A mature spindle with the same orientation of fibres; the chromosomes, however, have fused into large masses.

Fig. 15. A later stage, probably metakinesis.

Fig. 16. A portion of a cell-plate (see text for further explanation).

Figs. 17-22 pertain to nuclei of vegetative cells.

Fig. 17. Nucleus of an epidermal cell not far from the growing point, in the resting stage; the centrosphere is present, but the kinoplasm occurs in smaller quantities than in reproductive cells.

Fig. 18. Nucleus is prophase, showing chromatin spirem and large nucleolus.

Fig. 19. Spindle with chromatin in equatorial plate; centrosomes very distinct, but no polar radiations; the entire nuclear membrane is present.

Fig. 20. Similar to the preceding, but from a larger cell; the nuclear membrane (not drawn) had partly disappeared.

Fig. 21. Anaphase; the chromosomes are crowded into a mass so that the limits of the individuals are scarcely recognizable; the centrosomes are smaller than at an earlier stage.

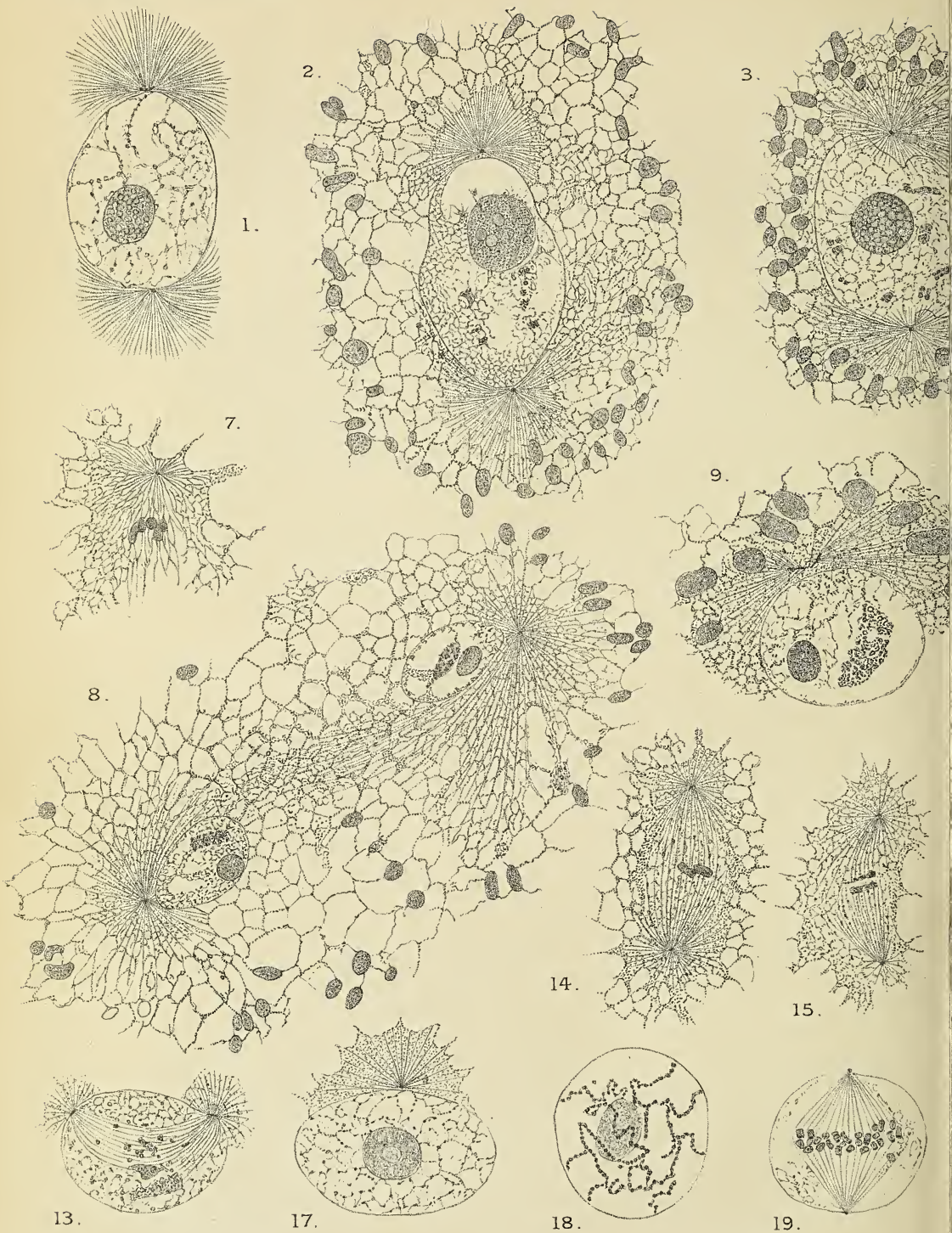
Fig. 22. An epidermal cell of the thallus. The daughter nuclei are provided with membranes, and the centrosomes, with fewer radiations, are present; on either side of the region formerly occupied by the connecting fibres cell-plate rudiments are seen. The connecting fibres have been replaced by a network which stains as kinoplasm.

Fig. 23. A germinating tetraspore. The centrospheres are on nearly opposite ends of the nucleus, which is structurally in the resting stage. The framework of the cytoplasm is more or less radially disposed about the nucleus.

Fig. 24. Daughter nucleus, resulting from the first division in the tetraspore after cell-division is complete. The nucleus is in the resting stage, yet the centrosphere shows that the karyokinetic activity of the next mitosis has begun. The persistence of the centrosome here is unquestionable.

Fig. 25. A nucleus taken from an enlarging epidermal cell, which gave an indication of development into a tetrasporangium.

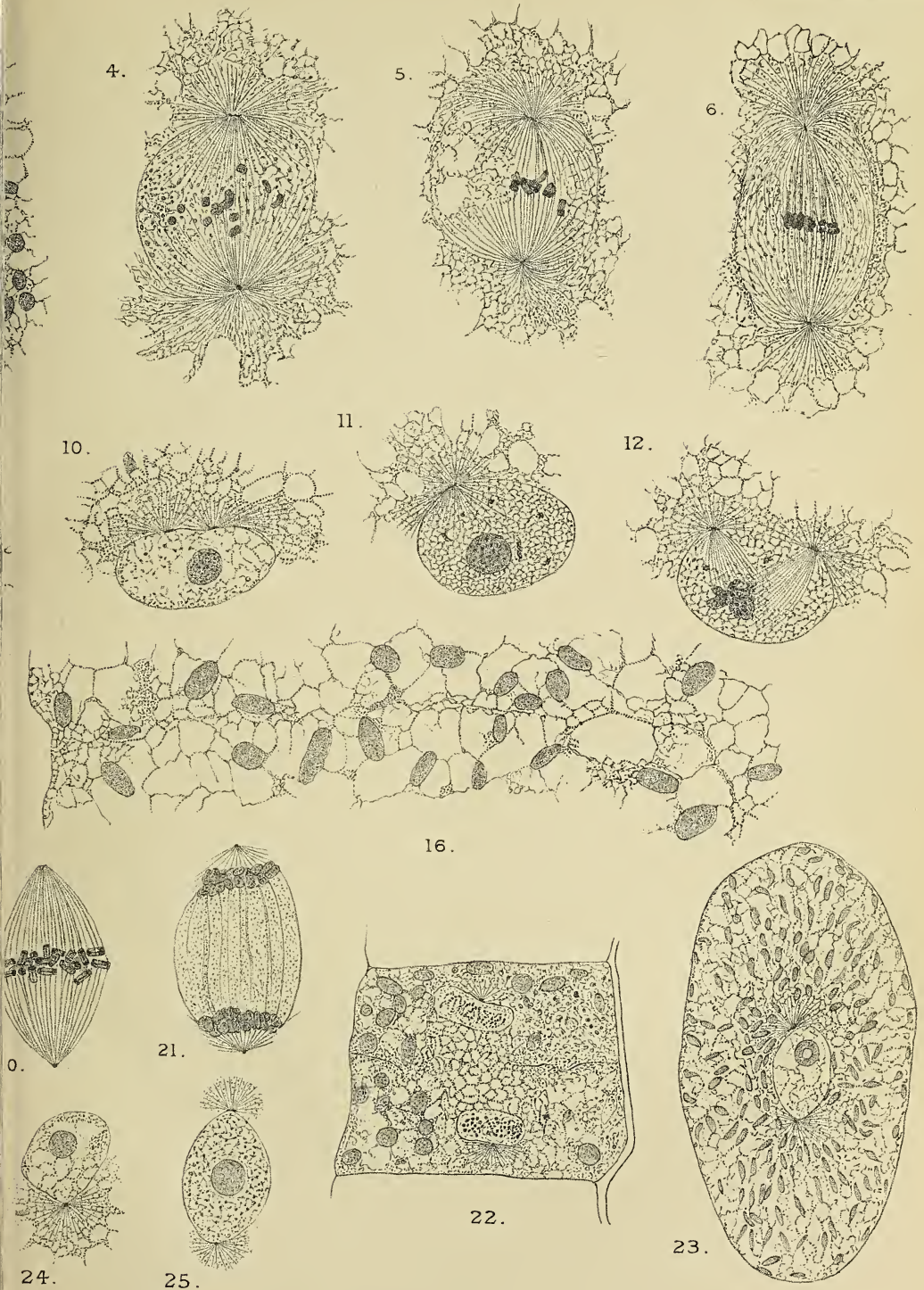




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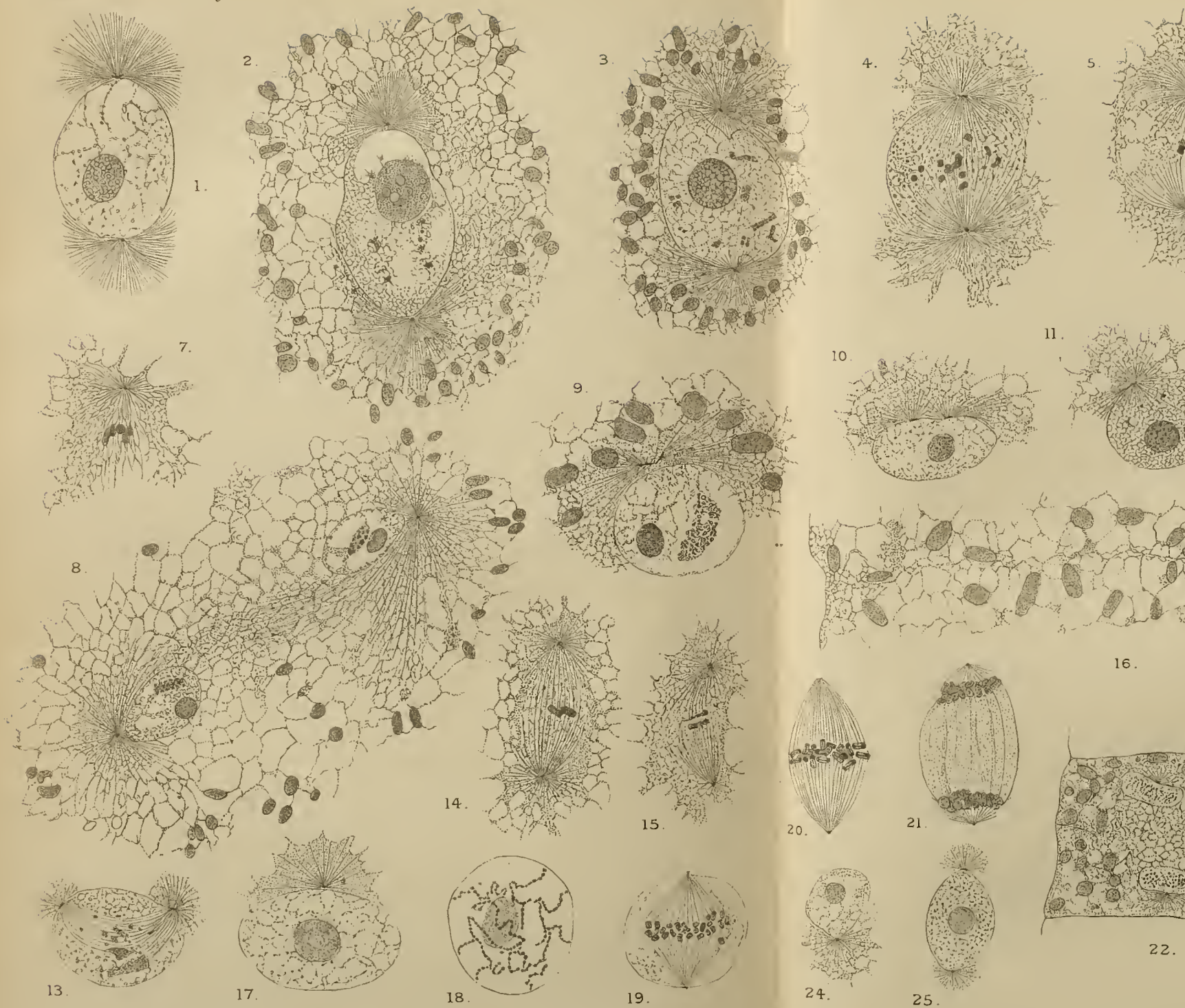
MOTTIER. — ON NUCLEAR AND CELL DIVISION IN DICTYOTA.











D.M. Mottier del.

MOTTIER. — ON NUCLEAR AND CELL DIVISION IN *DICTYOTA*.





# Observations on Latex and its Functions<sup>1</sup>.

BY

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With Plate XII.  
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DURING the year 1898 and the early part of 1899, while engaged in Ceylon as temporary scientific assistant to Mr. Willis, the Director of the Royal Botanic Gardens, Peradeniya, I was occupied in investigations on caoutchouc-yielding trees, chiefly *Hevea brasiliensis*, Müll.-Arg., (Para Rubber), and *Castilloa Markhamiana*, Markham, (a Central American rubber-tree)<sup>2</sup>. The principal results of this economic work are contained in one of the circulars<sup>3</sup> of the Royal Botanic Gardens, Ceylon, which is intended primarily for those interested in rubber-cultivation.

<sup>1</sup> A short abstract of this paper was read to the British Association, Sec. K, Dover, 1899; see Ann. of Bot., Dec., 1899.

<sup>2</sup> The *Castilloa* trees introduced into Ceylon in 1876 have hitherto been considered to belong to the species *C. elastica*, the well-known Mexican rubber-tree. They have recently been shown to belong rather to a closely allied species—perhaps hardly more than a variety of *C. elastica*—which is found in Panama and known as *C. Markhamiana*, Markham (not Collins). See Willis, Panama Rubber (*Castilloa*), R. B. G. Ceylon, April, 1899.

<sup>3</sup> Caoutchouc or India-rubber, its origin, collection, and preparation for the market, &c. Circular, R. B. G. Ceylon, June, 1899, pp. 105-168.

The purpose of the present paper is to draw attention to some of these observations and experiments with regard to their general botanical interest ; to describe other observations bearing on latex ; and to conclude with a few remarks and suggestions on the origin and functions of laticiferous tissue.

A word of apology is perhaps needed for the somewhat disconnected nature of the observations, which are, as it were, the outcome of what was fundamentally an economic study of rubber-plants.

There was not time to carry out more detailed work on the function of latex on the spot, and many of the matters touched upon can no longer be prosecuted at home with the same ease.

All remarks, unless distinctly stated to the contrary, apply to rubber-plants growing in Ceylon. Some of these may seem not altogether to tally with observations made on these plants elsewhere, thus admitting the possibility of the latex changing its character when the plants are moved from their natural habitats.

The paper is divided into seven parts, each dealing with a more or less distinct feature relating to latex, and concludes with remarks on its functions :—

- I. Proteids and the coagulation of latex.
- II. Oxydases in latex.
- III. The carbohydrates of latex.
- IV. Difference in properties between the latex of young and old organs of the same plant.
- V. The effect of wounding on the flow of latex.
- VI. A peculiarity in the exudation of latex from the severed base of the petiole of *Hevea brasiliensis* and *Plumiera acutifolia*.
- VII. A special laticiferous system in the developing seed of *Hevea brasiliensis*.



# I. PROTEIDS AND THE COAGULATION OF LATEX.

Coagulation of latex is now known to be brought about by the proteid contained in it passing from a soluble to an insoluble state, whereby the particles of caoutchouc, &c. in suspension are gathered together into clots; or, as Biffen graphically puts it in a paper<sup>1</sup> on the subject, the coagulating proteid 'gathers up the rubber particles in the same way as the white of an egg gathers up particles in suspension when clotted for the purpose of clearing jellies.' Consequently the conditions of coagulation depend upon the kind of proteid present in the latex. If it be globulin or albumen, then clotting should be brought about readily by heating; if albuminate, by neutralization.

*Hevea brasiliensis*. The proteid of this latex is said to be albumen, as is assumed in Biffen's paper<sup>2</sup>. Its coagulation has been studied in some detail in Ceylon, primarily for the purpose of devising the best means of preparing commercial indiarubber by the clotting method. The results suggest rather an albuminate than albumen as the particular proteid present.

The latex, such as is collected from incisions in the trunk of the tree, mixes in all proportions with water, and as it is too thick for experimental purposes when pure, it has usually been employed diluted ten times with water. This diluted latex is not clotted by heat—no change takes place in it after boiling for several minutes; it shows a great contrast in this respect to the latex of *Manihot Glaziovii*, Müll.-Arg. (Ceara Rubber), which in the diluted form clots readily when boiled, and fairly quickly in the cold. This latter latex contains a globulin.

If a trace of acid be added to the hot *Hevea* latex, clotting takes place immediately; on the other hand small quantities of alkalies postpone the coagulation indefinitely. It is not, however, necessary to add the acid to the hot latex; it brings

<sup>1</sup> Biffen, *Annals of Botany*, xii, June, 1898, p. 170.

<sup>2</sup> loc. cit., p. 170.

about the coagulation quite as completely, but not so quickly, in the cold. The approximate weight of acid required to completely coagulate 100 cubic centimetres of pure latex has been worked out for the following acids:—

Sulphuric acid	0.1	gram	clots	100	c.c.	latex.
Hydrochloric acid	0.1	"	"	"	"	"
Nitric acid	0.3	"	"	"	"	"
Acetic acid	0.95	"	"	"	"	"
Oxalic acid	0.2	"	"	"	"	"
Tartaric acid	0.25	"	"	"	"	"
Citric acid	0.5	"	"	"	"	"

The first two are thus seen to be the strongest coagulators, while acetic acid is the weakest, nearly ten times as much being required.

Two special points are to be noticed regarding this acid coagulation.

In the first place, the quantity of acid needed depends only on the amount of pure latex present in the liquid to be clotted. A certain weight of acid is required to completely coagulate 100 cubic centimetres of latex, no matter whether this be diluted to five or ten times its bulk. In other words, doubling the dilution halves the acid for a given volume, e.g., 100 c.c. of liquid containing 5 c.c. of pure latex requires half the amount of acid necessary for 100 c.c. of liquid containing 10 c.c. of latex. The latex can be diluted to any extent, and yet its particles of caoutchouc are capable of being collected together into clots by the addition of the necessary quantity of acid. This was even done for latex diluted 2,000 times.

In the second place, if the acid be added to excess, above a certain amount, coagulation ceases to be complete. By complete coagulation is meant the removal from suspension of all the globules of caoutchouc, so as to leave the liquid quite clear. The range for complete or nearly complete coagulation is very small with all the acids employed, except acetic. With sulphuric acid, for example, the amount can

hardly be doubled without interfering with the coagulation, whereas with acetic it can be increased some four times, before the residual liquid shows turbidity.

The following reasons are suggested for this behaviour of *Hevea* latex towards acids. The latex is slightly alkaline. The proteid is of such a nature as to be insoluble in neutral solution, but soluble in alkaline or acid media, i.e. it is an alkali-albumen. When the alkalinity is neutralized by the necessary amount of acid, the proteid comes out of solution and produces with the globules of caoutchouc the clots of rubber. If excess of acid be added, then the proteid remains in solution, being now in an acid medium. The acid required for coagulation bears a definite ratio to the quantity of pure latex only, no matter what its dilution may be, because the alkalinity is not altered in amount by this dilution. Acetic, being a weaker acid than the others, does not bring about the changes so rapidly.

The effect on this latex of several saline solutions has also been tested, viz.—sodium chloride, alum, ammonium sulphate, magnesium sulphate and mercuric chloride (corrosive sublimate). The last-mentioned has the strongest and most complete coagulating power on the latex. With 0.3 grams and upwards per 100 c.c. of pure latex, all the caoutchouc is separated by it from the diluted latex. This is what might be expected, since this salt is one of the strongest precipitants for proteids. Magnesium sulphate is the next best coagulator. A one per cent. solution brings about a fairly complete coagulation. Ammonium sulphate has to be added until as much as 5 per cent. is present, before coagulation is anything like complete. Sodium chloride never brought about a complete coagulation; whatever its strength the residual liquid was always quite milky. Alum was more effective. With an amount between 1.5 per cent. and 3 per cent., nearly complete coagulation was brought about, but above and below this the process was incomplete.

The amount of proteid present in this *Hevea* latex is considerable. By analyses kindly made for me by Mr. Kelway



Bamber, resident in Ceylon, by means of Kjeldahl's wet method, the amount of nitrogen found calculated out to about 2 per cent. of proteid.

*Castilloa*. The proteid of the latex of *Castilloa elastica* has also been investigated to some extent by Biffen<sup>1</sup>. He found that the latex gives an acid reaction, and that on the addition of a little alkali it is coagulated. This he considered to be due to the nature of the proteid which exists as acid-albumen in the latex; on neutralization it comes out of solution and gathers together the caoutchouc particles into clots.

Now the latex of the *Castilloa* introduced into Ceylon (*C. Markhamiana*) does not behave like this. On the very gradual addition of alkali to the latex or to the filtrate<sup>2</sup> of the latex no coagulation or precipitation occurs. Alcohol causes a coagulation of the latex and a copious precipitate in the filtrate, which is quite soluble again in water. Proteid is present in considerable quantity, about 4 per cent. being indicated by analysis. Coagulation is brought about neither by acids nor by boiling. Thus it looks as if the proteid belongs to the class of albumoses. At any rate the type of *Castilloa* introduced into Ceylon differs in this respect strikingly from that of the true *Castilloa elastica* examined by Biffen.

When the latex of *Castilloa* is mixed with water and allowed to stand, in the course of an hour or two the caoutchouc particles have all floated to the top in the form of a thick cream. The diluted latex of *Hevea*, on the contrary, shows no sign of creaming, even when submitted to a low temperature. The difference is most likely due to the larger size of the caoutchouc globule in the case of *Castilloa* as compared with that of *Hevea*.

The presence in latex of these most important plastic substances, proteids, is perhaps one of the strongest facts in support of the view that the laticiferous system takes part

<sup>1</sup> Biffen, loc. cit.

<sup>2</sup> The liquid part of the latex without the globules of caoutchouc.

in nutritive functions, either as a conductor or a storer of albuminous matter<sup>1</sup>. A thorough study of the proteids of latex is much needed. Such interesting work as that of Green<sup>2</sup>, done on latices which had been kept for some time, has not the same value as examinations of milk freshly drawn from the tree, owing to the liability of these complex nitrogenous bodies to change. It is a piece of research peculiarly fitted for the tropics, where laticiferous trees, which freely yield their milk, abound. The few laticiferous plants of temperate climes are mostly herbs, consequently the collection of their milk for chemical purposes is very tedious and unsatisfactory.

The latex of *Hura crepitans*, L. (Sandbox tree) is one well worth attention. It exudes copiously from a wound in the trunk and appears to contain abundant proteid, which seems to be largely globulin.

## II. OXYDASES IN LATEX.

Several latices, which are pure white when they first issue from a wound in the plant, rapidly darken on exposure to the air. This is due to the presence of an oxidizing ferment or oxydase, which, with the aid of the oxygen of the air, acts on some constituent of the latex, changing it to a deep brown colouring matter.

The latex of *Castilloa* is a good example. It rapidly darkens on exposure and dries to an almost black rubber. By creaming the caoutchouc particles can be separated from the dark beer-like liquid and made into a sheet of nearly colourless rubber. By quickly heating the collected latex, the darkening is arrested, owing to the destruction of the enzyme.

The latex of *Hevea* collected from the tree-trunk does not darken at all on exposure to the air, and provided that moulds and putrifactive organisms are kept away, rubber

<sup>1</sup> Sachs, Physiology of Plants, p. 362. Pfeffer, Physiology of Plants, p. 581.

<sup>2</sup> Green, Proc. Roy. Soc., 1886, No. 242.

prepared from it remains indefinitely of a light colour. On the other hand, the latex from the wall of the unripe capsule (fruit) changes on exposure, from milk-white to black. The darkening is wholly prevented, if the latex is quickly subjected to heat. No doubt there is an oxydase present in the latex of the capsule. Sometimes, but by no means always, the changing latex from the capsule shows an intermediate brick-red colour, before the blackening sets in. A peculiarity of this reddish colour is that it disappears on heating, whereas the black colour is permanent. The latex made to exude from young shoots sometimes blackens, but not always, whereas the blackening of the latex from the capsule is without exception in my experience. Oxydases, as a rule, show their presence more often in young organs than in mature ones.

### III. THE CARBOHYDRATES OF LATEX.

Sugar in variable proportions has been shown to be of frequent occurrence in latex. The little—0.3 to 0.7 per cent.—found in the trunk-latex of *Hevea* seems at all times to be cane-sugar. Examinations made at different periods of the year, thirteen in all, showed no reduction of Fehling's cupric reagent, till after heating with an acid. The latex of *Manihot Glaziovii*, examined on three separate occasions, behaved similarly. Tannin is absent from both of these latices.

May not some of the sugar contained in the collected latex come from the surrounding injured tissues, and not be originally present in the laticiferous tubes? I have noticed that in certain species of *Ficus*, the first two or three drops of latex from an incision in the stem taste much sweeter than those which exude later. A few experiments regarding this point were made on the latex of *Hevea*. From each incision the first latex exuded was collected separately from the next and so on. The sugar was then estimated in these different portions. Time did not allow of these experiments being more thoroughly carried out, but in such as were done the



results indicated most sugar in the latex which first trickled from the wounds, suggesting that the latex in oozing out had carried with it saccharine sap from the surrounding injured cells.

Biffen<sup>1</sup> has shown that the amount of sugar in latex is more in that collected from the plant in the late afternoon than in that in the early morning, the inference being that the laticiferous tubes receive the sugar arising from assimilation. Considering that the quantity is small, on an average about 1.5 grams of glucose per 100 c.c. of latex, it is quite possible that a considerable portion may have come from the adjacent injured tissues, such as the parenchyma and phloem-elements.

One of the most peculiar features connected with latex is the occurrence of the well-known rods of starch in the laticiferous tubes of *Euphorbia* and allied genera. Does it mean that these tubes serve as channels for the conduction in a solid form of the carbohydrate elaborated in the leaf?

It is an attractive view, and one which received a certain amount of support from the work of Treub<sup>2</sup> some years ago. He found that after darkening portions of the stems of succulent species of *Euphorbia*, the starch-rods had wholly or largely disappeared from the darkened areas. His results, however, are somewhat vitiated by the long duration of the darkening—three to five weeks.

Schimper's<sup>3</sup> experiments, on the other hand, show that the laticiferous tubes are not depleted of their starch in the dark. He found that the darkening of the leaves of *Euphorbia Peplus*, *E. Lathyris*, and *E. heterophylla* made, as a rule, no appreciable difference in the starch in the milk-tubes, whereas the starch disappeared wholly from the mesophyll of the leaf. He also incidentally mentions the fact that the dead leaves of *E. Lathyris* and *E. Myrsinites* have their tubes still full of starch.

<sup>1</sup> Biffen, *Annals of Botany*, xi, 1897, p. 338.

<sup>2</sup> Treub, *Ann. du Jardin Bot. de Buitenzorg*, iii, 1883, p. 39.

<sup>3</sup> Schimper, *Bot. Zeit.*, 1885, pp. 771-779.

Groom<sup>1</sup> confirms Schimper's work, that darkening does not make the starch disappear from the laticiferous tubes, and mentions that in *Euphorbia Peplus* the tubes contain starch till the death of the plant.

I find that these starch-rods are still present in the yellow and fallen leaves of such of these plants as have been examined, viz. *Euphorbia pulcherrima*, *E. Bojeri*, *E. Rothiana*, *Pedilanthus tithymaloides*, *Hura crepitans*, *Excoecaria bicolor*, *Sapium biglandulosum*; and appear to be here as numerous as in the mature green leaves. Since my return to England I have investigated the dead and withered leaves of *Euphorbia Lathyris* and *E. graeca*, and find the starch-rods quite evident in them.

It looks then as if it is a general rule for the starch to be left in the laticiferous tubes of the dying leaves. On this account, as well as from the fact that these starch-rods are well formed in leaves not yet mature, it appears as if they are produced once for all in the tubes as these differentiate, and exist throughout the life of the organ, having nothing directly to do with carbon-assimilation.

It is worth mentioning here that starch often remains in the guard-cells of the stomata as well as in the laticiferous tubes of dead leaves, whereas the rest of the leaf-tissue is, as a rule, free of starch: hence it might be argued that, as no doubt the starch is used by the guard-cells and reformed again and again, the starch of the laticiferous tube is also used and reformed again. This of course may possibly be the case. The point to emphasize, however, is that the starch seems to have no direct connexion with assimilation, and that the tubes can hardly be considered as starch conductors or storers. If it has any nutritive value, then it is for the use of the tubes themselves.

<sup>1</sup> Groom, Annals of Botany, iii, 1889.

## IV. DIFFERENCE IN PROPERTIES BETWEEN THE LATEX OF YOUNG AND OLD ORGANS OF THE SAME PLANT.

While investigating the latex from young stems and leaves of certain indiarubber trees, with the object of testing the feasibility of extracting commercial rubber from them, it was found that in several instances the 'rubber' obtained was quite different from that got from the trunk and main branches. This is an important point relative to the production of caoutchouc from young organs.

In the case of *Hevea*, the rubber collected from the young stems and leaves, as well as from the unripe capsules, is somewhat adhesive, and has less elasticity and strength than that from the trunk.

In the *Castilloa* introduced into Ceylon, the latex from the stems bearing leaves, as well as from the leaves themselves, moulds between the finger and thumb into a very sticky substance, wholly unlike the caoutchouc-containing latex of the trunk. It dries to a brittle material, which becomes viscous when warmed. The quality of the rubber from stems of this *Castilloa*, 12.5 to 25 c.m. in circumference, was likewise tested; it seemed to have properties intermediate between that of the shoots and the trunk, being slightly sticky and somewhat deficient in elasticity. From all that I have heard, the true *Castilloa elastica* does not exhibit this distinction between the shoot and trunk.

The climbing rubber-plants *Landolphia Kirkii* and *Urceola esculenta* show a similar difference between the latex from the shoot and that from thick stems.

*Ficus elastica* also exhibits this peculiarity<sup>1</sup>. Attention was called to this in *Ficus* as far back as 1839 by Weinlung. He called the substance 'viscin,' and considered it intermediate between resin and caoutchouc.

Perhaps in the foregoing plants the laticiferous tubes

<sup>1</sup> See Weiss, Trans. Linn. Soc. iii, 1892, p. 243; also Seeligman, Le Caoutchouc et la Gutta-percha, 1896, p. 91.



formed in primary growth have their globules in suspension in the latex, of a different composition from those arising in the secondary tissues.

In many plants this so-called viscin seems to occur throughout the laticiferous system, e.g. the common bread-fruit (*Artocarpus incisa*) and jak (*A. integrifolia*)—trees of the tropics.

Most likely there are bodies which do not come within the categories of caoutchoucs and guttas, and yet are hydrocarbons with the same percentage composition. Probably some of these viscous substances are such. Also it appears probable that all caoutchoucs are not identical, and that when prepared as pure as possible from the latex, as by the ingenious centrifugal method of Biffen, it may be found, for example, that the caoutchouc of *Hevea* has slightly different properties from that of *Castilloa*.

#### V. EFFECT OF PREVIOUS WOUNDING ON THE FLOW OF LATEX.

An important fact connected with the tapping of *Hevea* trees, and a remarkable one from a botanical point of view, is that wounding the bark causes a greater flow of latex from subsequent injuries.

Mr. Willis<sup>1</sup>, in his experiments on the yield of these trees, found that the weight of rubber obtained from the second tapping of a series of trunks was about double that obtained from the first incisions. Further experiments have shown that this increase in weight is due to a much greater flow of latex, from fresh wounds in a tree recently tapped, than in one hitherto intact.

One of our experiments indicated that it is possible to increase the quantity of latex to as much as seven times that obtained at the first tapping, using the same number and kind of incisions on each occasion. This fact we have found

<sup>1</sup> Willis, Rubber Cultivation in Ceylon. Circular R.B.G. Ceylon, Jan. 27, 1898.

subsequently to be well known among the rubber-tappers of the Amazon valley, the home of *Hevea brasiliensis*. However, it seems, up to this, hardly to have been mentioned in the literature on the subject of rubber-tapping, although it is one of the most important points connected with the yield of caoutchouc from *Hevea*.

The increased quantity of latex from a new incision in the bark made 10 to 15 centimetres distant from an old one, can hardly be due to the formation of new milk-vessels, since the wound-response and increase in question may be recognizable after the lapse of a single day.

This response to wounding seems an important point relative to the function of latex, and a very practical one regarding the tapping of Para rubber-trees; and it needs further investigation. It will also be interesting to see whether other rubber-trees, such as *Manihot Glaziovii* (Ceara rubber), behave in a similar manner.

Full details respecting the practical results of experiments on this wound-response are to be found in the Circular mentioned at the beginning of this paper.

#### VI. A PECULIARITY IN THE EXUDATION OF LATEX FROM THE SEVERED BASE OF THE PETIOLE OF *HEVEA BRASILIENSIS*, Müll.-Arg., AND *PLUMIERA ACUTIFOLIA*, Poir.

*Hevea brasiliensis*. The leaf is trifoliate, with a long petiole, slightly swollen where it joins the stem. The large lanceolate leaflets are attached by very short stalks to the petiole.

The following points may be noted regarding the flow of latex:—

1. When the petiole of a *mature* foliage leaf is cut or broken sharply across at its base (Plate XII, line A-B, Fig. 1), no latex exudes from the injured surface attached to the stem, whereas the surface of the detached petiole is immediately suffused with latex.

2. When the severance is made *quite close* to the stem

(line C-D in the diagram) the reverse takes place, and latex oozes out only from the stem side.

3. On cutting through the leaf-stalk higher up (such as E-F in the diagram), latex exudes freely from both surfaces.

4. By making the incision follow as closely as possible the place where the absciss-layer will eventually be formed (indicated by the broken line in the diagram), it is almost possible to sever the base of the petiole without any exudation of latex appearing on either surface.

5. In a leaf *which has not yet reached maturity*, these peculiarities are not observable; latex exudes from both surfaces alike, when the petiole is cut across at the base (such as A-B in the diagram).

6. When the base of the short stalk (petiolule) of the leaflet of a mature leaf is cut through, latex appears on both surfaces. These leaflets are disarticulated by means of absciss-layers just as the petiole is, but do not resemble the latter in the peculiarity of the exudation of latex.

These observations point to an obliteration of the cavities of the laticiferous vessels at the region of the petiolar base, on the leaf attaining maturity. Since the place of interruption to the flow of latex appears to correspond with the position of the absciss-layer, the idea naturally suggests itself that the special cells composing this layer are formed on the maturity of the leaf, and cause the closure of the laticiferous vessels at this point. A microscopic examination, however, shows no definite layer of cells at all in this position. The absciss-layer only seems to be formed about the time that the leaf changes colour previous to its fall.

There are two marked differences between the microscopic appearances of the young and mature leaf-base. As the leaf assumes its adult condition, certain cells making a more or less broken layer across the base of the petiole become sclerosed to form 'stone' cells; there is also a marked deposition of cluster-crystals right across this region. Such structural changes suggest a closure of the laticiferous vessels



at this point, due to the pressure upon them of these altering cells.

About one hundred laticiferous plants have been tested regarding the flow of latex from the cut petiolar base, and in only one other plant, *Plumiera acutifolia*, has a case been found at all as striking as that of *Hevea*.

*Plumiera acutifolia*. The flow of latex from the cut petiole takes place precisely as in *Hevea*. Unlike it, however, a microscopic examination reveals no special structural changes in the base of the petiole on the leaf attaining maturity. There is no formation of 'stone' cells or deposition of crystals, the only difference being that the laticiferous tubes are less marked in the adult than in the young leaf.

*Plumiera rubra* behaves similarly, but *P. obtusa* does not exhibit this peculiarity; latex issues from both surfaces when the base of the petiole is cut across.

Contrasting the two cases of *Hevea* and *Plumiera* it looks as if the interference with the flow of latex, at the base of the petiole, may be brought about, not by any special form of cellular tissue closing the tubes, but rather by the pressure of the ordinary parenchyma at a region destined to be that of the absciss-layer.

Microscopic examinations have failed to show any definite obliteration of the cavities of the laticiferous vessels, crossing from the leaf to the stem. They seem, however, to be more crushed here than elsewhere in the petiole.

The severing experiments are difficult of explanation on any other supposition than that of the obliteration of the cavities of the tubes, unless it be that the block occurs in the tubes themselves, as by a coagulum forming in the latex, or by an ingrowth of the walls of the tubes.

Since this phenomenon is so exceptional amongst laticiferous plants, it may perhaps be looked upon as merely accidental, and an extreme case of the general tendency for laticiferous tubes to be somewhat crushed in mature tissues.

Nevertheless, it seems to me that this peculiarity affords to some extent an argument against the view that the latici-

ferous tubes act as agents for the conveyance from the leaf of plastic substances formed in the mesophyll, at least as far as these two plants are concerned.

#### VII. A SPECIAL LATICIFEROUS SYSTEM IN THE YOUNG SEED OF *HEVEA BRASILIENSIS*, Müll.-Arg.

If laticiferous tubes have the function of conducting food-materials to growing organs, one might expect to find a rich development in the fibrovascular bundles going from the placenta to the ovule. On this account I was led to examine the developing seeds of *Hevea*.

The gynoecium is of the typical Euphorbaceous type, consisting of three carpels united into a trilocular ovary with a single suspended anatropous ovule in each loculus. After fertilization the ovary wall becomes differentiated into two parts, an inner portion composed of cells, which lengthen greatly in the radial direction, and lignify to form the hard wall of the capsule, and an outer layer which retains its parenchymatous condition and in which a rich laticiferous system is developed. The laticiferous tubes are not so extensive in the septa and central column bearing the placentas. The funicle and raphe of the developing seed are almost devoid of laticiferous tubes. A number of ovules and seeds have been examined in various stages of development, and only very occasionally has a laticiferous element been seen in these regions. The main fibrovascular bundle passing through the funicle and raphe to the chalaza is thus, as a rule, not accompanied by laticiferous vessels, at any rate by tubes containing caoutchouc and similar substances.

While examining the developing seed, a somewhat singular development of laticiferous elements was discovered just beneath the inner limiting layer of cells of the inner integument. These are absent from the ovule at the flowering stage, and only arise sometime after, when the capsule has reached a considerable size.

Figures 2 and 3 show the situation and general arrangement

of this laticiferous system in a seed, which has nearly reached its full size, but which is still far from maturity. A sheath, as it were, of laticiferous tissue forms around the nucellus, extending right from the chalaza to within a short distance from the micropyle. This is marked in thick irregular lines in the figures, one of which represents a median longitudinal, and the other a transverse section of the young seed.

The *Hevea* trees in the Peradeniya Gardens from which the material was collected, flower during April. Some capsules taken on May 5, which had grown to the size of large peas, showed no sign of this laticiferous system in the inner integument. The system at this early stage had not commenced to be differentiated.

A second lot obtained on May 13, with young seeds 5 to 6 millimetres long, revealed the commencement of this laticiferous system. Groups of parenchymatous cells situated, as a rule, about one or two rows of cells external to the inner limiting layer of the inner integument, increase considerably in size—to four or five times that of the neighbouring cells. Their walls thicken somewhat and their contents assume a more coarsely granular condition (Fig. 4). These, the laticiferous cells, appear first about the chalaza and develop more rapidly in the half of the seed along which the raphe runs; indeed the production seems finally to be greater in this half than in the other. The walls between groups or rows of these cells partially break down, and processes grow out from many of them.

At a later stage (middle of June) this laticiferous system had reached about its full development. The processes are now a conspicuous part of the system; they branch a little. When a young seed at this stage is cut across, latex oozes out around the nucellus, or rather around the developing endosperm, since the former is now much crushed.

This laticiferous system then of the inner integument consists of a number of cells communicating one with another, due to the partial dissolution of the intervening walls, and of unsegmented processes emitted from these cells (Figs. 5 and 6). Such a laticiferous system suggests a blending



together on a small scale of the articulate and inarticulate types.

No such laticiferous tissue is developed in the young seed of the somewhat closely allied rubber-tree, *Manihot Glaziovii*. The developing seeds of three other members of the order have been examined, viz. *Croton tiglium*, *Jatropha multifida* and *Euphorbia Lathyris*, without finding any such system.

It will be instructive to extend the investigation to other genera of the laticiferous Euphorbiaceae, to ascertain whether any more cases like that of *Hevea* are to be found, likewise to see whether this laticiferous system of the seed-coat of *Hevea* may be considered as a new production or as vestigial in the evolution of the group, as well as to endeavour to discover what function, if any, it may perform here.

This laticiferous system just described is interesting from another point of view. Scott<sup>1</sup>, who has investigated the laticiferous tissue of the vegetative parts of *Hevea brasiliensis* and *Manihot Glaziovii*, showed that the tubes are true vessels, formed by the breaking down of the septa of rows of cells, and that these do not give out conspicuous processes. On the other hand, in the young seed of this plant, it has just been pointed out that the processes or unsegmented outgrowths from the cells are an important item in the construction of this laticiferous system.

#### REMARKS ON THE ORIGIN AND FUNCTION OF LATICIFEROUS TISSUE.

The morphology of the laticiferous tissue of the Euphorbiaceae is of much interest. When De Bary published his Comparative Anatomy of the Phanerogams and Ferns, the Euphorbiaceae were considered as including only plants with the inarticulate type of laticiferous tube.

Scott<sup>2</sup> in 1884 and 1885 showed that *Manihot* and *Hevea*, members of this order, had, however, an articulate laticiferous

<sup>1</sup> Scott, Quart. Jour. Micro. Soc., Vol. xxiv, 1884, and Jour. Linn. Soc., Vol. xxi, 1885.

<sup>2</sup> Scott, loc. cit.

system, and put forward the view that laticiferous tissue has been evolved independently along two distinct lines in the Euphorbiaceae.

The elaborate work of Chauveaud<sup>1</sup> in 1891 further complicated the story of the origin of the laticiferous apparatus in the order. He finds in such genera as *Aleurites* and *Fatoupha*, an embryonic inarticulate system replaced by an articulate one, and was driven to the conclusion that in this group the inarticulate system is the more primitive.

There is still room for a considerable amount of anatomical work on the laticiferous elements of the Euphorbiaceae, in order that the origin and development of the two systems may be traced.

It seems to me possible that an articulate system may give place to an inarticulate one by the gradual substitution of elongation and branching of the laticiferous cells for previous fusion of them. On the other hand, it might be simpler to regard the two systems as of independent origin from simple laticiferous cells, if it were not that the results of the work of Chauveaud quoted, hardly allow of this.

The origin of laticiferous tubes from secretory sacs containing tannin, resin, &c., is strongly suggested by such groups as the Papaveraceae and Aroideae, where there is a gradual transition from secretory cells to true vessels. Again, laticiferous plants as a whole are devoid of other secretory reservoirs.

Assuming that secretion, or the holding of substances of no further nutritive value, is the primary function of laticiferous cells, why is it that in certain groups of plants these cells, either by great elongation and repeated branching, or by the formation of vessels, have been changed into a complicated system of communicating tubes? Surely to perform some additional function or functions.

A conducting function is the one which naturally suggests itself. Since extracted latex contains such valuable food-stuffs as proteids and carbohydrates, the view of the tubes

<sup>1</sup> Chauveaud, Thesis. Paris, 1891.

being channels for the transport of these materials is one which has received much consideration. The early development of the system in the embryo and young organs, as well as its association with the sieve-tubes, are brought forward in favour of this theory. It has even been suggested that in certain plants they take the place of sieve-tubes, since these latter diminish in number as the laticiferous tubes increase.

Another hypothesis put forward is that latex functions as a protection to the plant, either by closing wounds or by checking the ravages of insects. No conclusive evidence has been brought forward to show the likelihood of the laticiferous system of any plant having been evolved for such reasons. These plants seem just as liable to insect pests and fungus-diseases as others. In a tree like *Hevea* with thick latex, no doubt an injury to the bark is effectually closed by a plug of rubber, thus preventing, we may suppose, the entrance of fungus-hyphae, but to conclude that the latex has been elaborated for such a purpose is another matter.

If the formation of laticiferous tubes has been called forth in all plants possessing them to perform a common function, then I am inclined to think the idea of their serving as channels for holding water in reserve as one of the most plausible. Laticiferous plants are markedly characteristic of tropical regions, where transpiration is great. The development of a system of tubes running throughout the plant to be filled with water during the wet season and then to be gradually drawn upon during times of drought, is intelligible.

Warming, in a paper in the Botanical Gazette for January, 1899, entitled 'Vegetation of Tropical America,' mentions lianas and other plants of tropical forest and scrub as often laticiferous, and says, 'most likely latex serves several purposes, and one of them, I suppose, is to supply water to the leaves in times of need when transpiration becomes too profuse.'

From our experiments in Ceylon we found that the quantity of latex extractable from incisions in the trunks of *Hevea* trees varied considerably with the time of year, and seemed



to depend largely upon the available moisture in the soil. After heavy rain the exudation of latex is much more copious and thinner, looking as if the vessels had become surcharged with water.

As the necessity for a reserve of water increased, the laticiferous system would tend to become more extensive and more intimately associated with the surrounding tissues. The genus *Euphorbia* chiefly inhabits dry regions and is one of the richest in latex.

This view does not explain the proteid or starch grains of latex, yet, I think, it is one to be borne in mind in studying the rôle of latex in plants, and hitherto it has in the main been disregarded. If latex does serve as a water reserve, then perhaps it is chiefly valuable for the growing organs.

Our knowledge of the function of latex can hardly be regarded as having advanced as yet much beyond the domain of hypothesis. There is a considerable amount of work to be done before we can arrive at the true meaning of such a richly developed system of milk-tubes as that in an *Euphorbia*. We know very little about the metabolic processes which result in the production of the secretions—tannin, resin, caoutchouc. We are still very ignorant of the way in which the sieve-tubes with their companion cells deal with proteids, though it has been suggested that the laticiferous tubes might relieve them of part of their work.

The problem is one of much interest in physiological botany, and not without its direct practical bearing on the production of the commercial articles, indiarubber and gutta-percha. The solution of it may show that latex does not play such important parts in the plant's economy as has been claimed for it in the past.

## EXPLANATION OF FIGURES IN PLATE XII.

Illustrating Mr. Parkin's paper on Latex and its Functions.

The figures all refer to *Hevea brasiliensis*.

Fig. 1. Diagram of the attachment of the petiole to the stem (nat. size). *p.*, petiole; *s.*, stem; *b.*, axillary bud; *sc.*, scar of stipule. The rest is explained in the text.

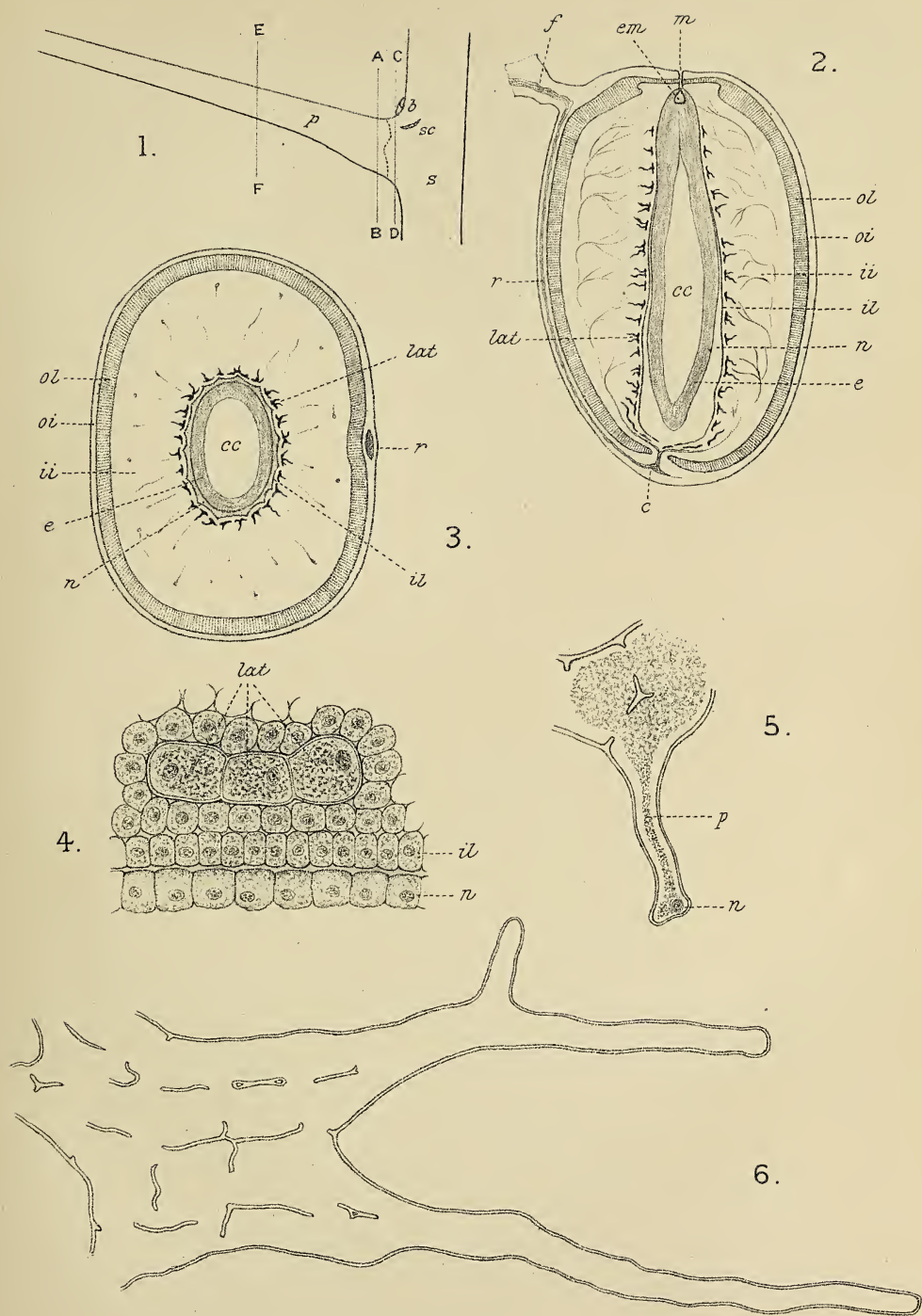
Figs. 2-6 refer to the laticiferous system of the young seed.

Figs. 2 and 3. Diagrams of median longitudinal and transverse sections respectively of young seed, which is nearly full size, but considerably off maturity ( $\times 3$ ). *lat.*, laticiferous tissue exaggerated in size; *f.*, funicle; *r.*, raphe with main fibrovascular bundle; *c.*, chalaza; *oi.*, outer integument; *ii.*, inner integument with fibrovascular bundles; *ol.*, outer limiting layer of cells of inner integument which lengthen greatly in the radial direction and sclerose to form the hard coat of the seed; *il.*, inner limiting layer of cells of the inner integument, external to which is situated the laticiferous tissue; *n.*, remains of nucellus; *e.*, developing endosperm; *em.*, embryo; *m.*, micropyle; *cc.*, central cavity.

Fig. 4. Early stage in the formation of the laticiferous tissue ( $\times 550$ ). *lat.*, three laticiferous cells differentiated from the surrounding parenchymatous cells; *il.*, inner limiting layer of cells of inner integument; *n.*, outer layer of cells of nucellus.

Fig. 5. A piece of the laticiferous tissue at a later stage ( $\times 550$ ); cells in communication, due to the partial dissolution of the intervening walls. *p.*, process; *n.*, nucleus.

Fig. 6. Portion of laticiferous tissue fully developed; cell-walls only represented, contents having been dissolved out by chloroform. Note the partial dissolution of the intervening walls of the laticiferous cells and the long processes given out. ( $\times 550$ .)



J. Parkin del.

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# A Fragrant 'Mycoderma' Yeast, *Saccharomyces anomalus* (Hansen).

BY

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With Plate XIII.



AT the beginning of August 1899, during the course of a series of experiments on the fermentation of ginger, a greyish-white floury film was frequently found on the surface of saccharose-Mayer solutions<sup>1</sup> to which, after sterilization, pieces of commercial ginger had been added. Before the formation of this film active fermentation had been taking place in the liquid, and a slight growth of a species of *Aspergillus* and other organisms had appeared. Towards the end of active fermentation the film made its appearance and rapidly covered the surface of the liquid: about the same time a pleasant fruity odour was apparent, in some cases so strong that the whole laboratory was scented with it. The growth of the mould was stopped by the development of the film, because the latter prevented access of oxygen to the former. On examination of a portion of this film under the microscope, it was found to be made up of a number of small oval and round Yeast-like cells. The organism in its appearance

<sup>1</sup> Pure saccharose 15 grams. Mayer's Solution 10 c.c.

and manner of growth seemed to be a form of so-called *Mycoderma*, with considerable resemblances in external characters to the ordinary *M. cerevisiae* and *M. vini*, but closer investigations showed that it is a very different organism.

Pure cultures were obtained by the following method:—a portion of the film was shaken up vigorously in a test-tube of sterile distilled water, and a drop of this liquid then poured into a test-tube of beer-wort gelatine<sup>1</sup>. From this a fractional series of six plate-cultures of beer-wort gelatine was made and allowed to grow. Then a second series, infected from a colony growing on a plate of the first series, was made, to ensure the purity of the culture.

A stock of the organism was obtained by making a streak-culture on a tube of beer-wort gelatine from a colony developed on a plate of the second series. In order to prevent contamination of the stock, a new stock-tube of beer-wort gelatine was always infected whenever the original stock-tube was opened to obtain a supply of the organism, and the new stock used for the next supply. Occasionally a new series of plates was made, infected from the stock-tube in order to check the results, and to ensure that the organism conformed to type, and a new stock made from a single colony on one of the plates.

#### MORPHOLOGY.

In general culture on beer-wort, dextrose-Mayer solution, or other nutrient solutions<sup>2</sup>, this Yeast forms, as a rule, a greyish-white film on the surface of the liquid. It has a very floury or powdery appearance, as if composed of innumerable greasy particles; and if the growth is very vigorous, the film becomes much wrinkled by mutual crowding of its units. Most of the cultures were made in Erlenmeyer flasks, and the Yeast had a curious habit of growing over the surface of the glass above the liquid up

<sup>1</sup> Unhopped Beer-wort 90 c.c. Gelatine 10 grams.

<sup>2</sup> See p. 230 for the constitution of these solutions.



towards the neck, like a greasy film on the moisture there found, the growth appearing somewhat mycelium-like in nature when undisturbed. A copious deposit of cells soon forms at the bottom of the flasks owing to gradual wetting and precipitation of the old cells: this deposit is white in colour at first, but becomes a dirty-brownish colour in old cultures. The liquid itself remains clear, when the film attains a good development.

#### CULTURE IN TUBES.

Streak-cultures were made on tubes of sloped beer-wort gelatine, and grew rapidly. A well-developed streak has in most cases a milky-white appearance. It is quite opaque, except that part of it which grows on the thin layer of gelatine at the upper part of the sloping surface, where the streak is semi-transparent and thin, and usually develops a mycelium-like fringe at its edge. The other portion of the streak is thick, moist, and fatty-looking, and its edges are more or less crenate and well-rounded. It does not grow down into the gelatine, but remains on the surface. In some cases when the gelatine dries owing to various causes, the streak has a different appearance altogether, being flat, glistening white in colour, eventually becoming dry and almost powdery, and marked with radial and concentric zones.

Beer-wort agar<sup>1</sup> has also been used, and the growth on this is even more rapid. Streaks similar to the fatty-looking streaks on beer-wort gelatine are produced.

#### PLATE-CULTURES.

*On beer-wort gelatine.* The forms of the colonies are by no means constant on different plates: on the same plate, however, as a rule they are fairly constant. This applies not only to plates of different series, but to plates of the same series, and will be explained below.

The colonies appear as small whitish dots. Those on the

<sup>1</sup> Agar 2 grams. Wort 90 c.c.

surface of the gelatine grow in the form of small domes, to about the size of a pin's head. Growth then takes place over the surface of the gelatine, and a thin flattened disc is produced. Sometimes it is circular in outline, glistening white in colour, and dry in appearance, marked by radial and concentric markings, and with regular edges (Plate XIII, Fig. 4). Sometimes it is like the preceding, except that it is semi-transparent, moister looking, more film-like, and with less regular edges. In other cases the colony is like an irregularly circular film, with crenate edges. semi-transparent, moist looking, with indications of radial and concentric zones (Fig. 3).

Colonies intermediate in character between the above forms have been observed. The form seems to be dependent on several different factors. The consistency of the gelatine has undoubtedly a great deal to do with the ultimate form. Plates were made with five per cent. gelatine instead of ten per cent., and the most usual form of colony produced in this case was the third described above. An abundance of moisture and higher temperatures ( $21-22^{\circ}\text{C.}$ ) tend towards the production of the same form. Colonies of the first form described have been observed to change into the other forms on increase of temperature.

*On lactulose-Mayer gelatine* the young stages of the colonies are similar to those on beer-wort gelatine. Instead, however, of the tiny dome-shaped colonies developing into flattened discs, as on beer-wort gelatine, they continue to grow in a dome-shaped manner, and in colour are glistening white. As they age, their surfaces are pitted with numerous tiny depressions, and at a later stage are fluted in a radial and concentric direction. They eventually become cone-shaped, or else develop a depression at the top of the dome. (See Figs. 1 and 7.)

In old colonies, as a rule, longitudinal splits are formed and they fall away into segments. Sometimes, however, they lose their white colour with age, becoming brown, and have a moist appearance, partially losing their shape. In a few

cases they changed to such a degree as to look like small drops of brown jelly. In other cases they changed into flat circular milky-white colonies, much resembling solid drops of stearin, and recalling the appearance of streak-cultures on beer-wort gelatine (see Fig. 8). All these later changes are due to slight liquefaction of the gelatine, accompanied by either a soaking in of the beer-wort, or some oxidation.

Plate-cultures made with dextrose-Mayer gelatine and saccharose-Mayer gelatine produce colonies of the same form as those in laevulose-Mayer gelatine.

*Saccharose*, *laevulose* and *dextrose* gelatine (relying on the traces of mineral substance in gelatine in the absence of other inorganic salts) were also used as media for plate-cultures. The colonies on each were similar in form. They grow as dots to the size of about a pin's head above the surface of the gelatine, but the growth is very slow, and then the formation of outgrowths like mycelia begins, and may continue until the central dot-like portion of the colony is surrounded by feathery radiations. See Fig. 6 showing colonies on saccharose gelatine.

*General observations on colonies in plate-cultures.*

The form of the colony seems to be dependent on the temperature, the amount of moisture, the crowding of the Yeast-cells and their vigour, the consistency of the gelatine, and the nature of the food-material. Old colonies have a tendency to produce mycelium-like outgrowths at their edges.

This matter would seem to be not without importance to the general question of the macroscopic appearance of colonies on plate-cultures. The typical form appears to be that of the raised, dry, chalky-white, brittle and even powdery wrinkled dome shown in the photograph (Figs. 1 and 7); but it is evident that any of the circumstances mentioned may so modify this that in extreme cases it would be difficult to recognize this organism from its plate-cultures.



*Culture in hanging drops.*

Cells grown under observation in hanging drops of beer-wort gelatine were ellipsoid or more or less ovoid in the adult stage. They were filled with clear, colourless protoplasm, and the extremely thin cell-walls were not distinctly marked off from the cell-contents. A single isolated cell may easily be overlooked on account of its transparency in the gelatine. At a temperature of 19–19.5°C. a single adult cell of the above form produced another similar adult cell in two hours by normal budding, the bud arising generally from the neighbourhood of the more pointed end. In the case figured (Fig. 9) the single cell had produced four similar cells in four hours, and in six hours seven cells. The daughter cells are at first round, but become usually more ovoid before producing another cell.

As a rule, after a colony of about eight cells was produced, the gelatine in the immediate neighbourhood was liquefied, with the result that the cells separated from one another, and the colony was thus broken up. Owing to the diminution of resistance as the gelatine softened, the buds separated from the mother cell much earlier than was the case when the gelatine remained unliquefied. These young separated buds bore a great resemblance to small *Torula*-like Yeasts. At a temperature of 15° C. the gelatine was not so rapidly liquefied, consequently larger colonies were found before separation, some containing a great number of cells. In hanging drops at this temperature about thirty-six hours old, the cells at the edges of the colonies have a tendency to grow out into a false mycelium, becoming longer and more rod-like in appearance. The colony then looks like a many-rayed star (Fig. 5), owing to the radiating series of branching cell-series to which I have throughout applied the term 'mycelium-like.'

In hanging drops a week old at 19° C. the cells have a sharply-marked cell-wall and are more or less vacuolated. Many of them contain bright refringent granules, and in some, spores were observed, as will be described subsequently.

## FILMS.

*Primary films*<sup>1</sup> are formed by this Yeast on the surface of every culture-solution in which it is able to grow. They differ in character and in rate of growth according to the constitution of the culture-solution.

On beer-wort in most cases the film begins to form in twenty-four to forty-eight hours at 28° C. and twenty-four hours later appears as a thick greyish-white, dry and powdery, greasy looking, much wrinkled film. Fermentation then proceeds actively, and the film is to a great extent broken up by the bubbles of carbon dioxide evolved. When active fermentation ceases the film re-forms, but not so strongly as before, and after a time disappears and is replaced by the secondary film. In a few cases no continuous film was formed until fermentation ceased. At 18° C. the formation of the film took place more slowly.

On dextrose-Mayer solution a film is produced similar to that on beer-wort. It is however better-developed, more wrinkled and of a whiter colour. Formation begins in twenty-four to forty-eight hours at 28° C. Its fate is similar to that on beer-wort.

On saccharose-Mayer and laevulose-Mayer solutions the films are like those on dextrose-Mayer. On lactose-Mayer solution the first signs of film formation appear in about forty-eight hours after infection at 28° C. In its early stages it looks like a few patches of fatty matter lying on the surface of the liquid. These patches increase in size and eventually fuse together, forming a complete film over the surface in five days after infection at the above temperature. It is very thin and somewhat fatty-looking, and is also semi-transparent. It gradually breaks up and disappears.

On maltose-Mayer solution a film begins to develop in

<sup>1</sup> By primary film is meant the film which forms before fermentation, as distinct from the veils (secondary films), which Hansen found were developed by many Yeasts after the culture has stood for some time. (See 'Jørgensen, Mikroorganismen der Gährungsindustrie,' 4th ed., 1898, p. 173.)

about forty-eight hours after infection at 28° C. When fully developed it has a greyish-white colour, is very thin, and has a powdery appearance. It is not wrinkled, but resembles early stages in the development of the films on beer-wort and dextrose-Mayer.

On dextrin-Mayer solution after twenty-four hours at 25° C. the film begins to be visible. Twenty-four hours later it is thick, much wrinkled and of a grey colour, being identical in appearance with the films on beer-wort. It gradually breaks up and eventually disappears altogether.

On soluble starch in Mayer's solution a thin white powdery-looking film is formed in about seven to ten days at 28° C. The film is not usually well developed on the liquid, but spreads vigorously over the surface of the flask in a somewhat mycelium-like manner.

On examination under the microscope, the primary films are seen to be made up of Yeast-cells, actively budding, enclosing in the spaces between the cells numerous gas-bubbles. These bubbles adhere very tenaciously to the cells, and appear to be concerned in preventing the wetting and sinking of the film in water.

*Secondary films* are formed by this Yeast on those solutions in which it is capable of inducing alcoholic fermentation, viz. on beer-wort, dextrose, laevulose and saccharose-solutions. They begin to make their appearance some time after active fermentation has ceased, and after the primary film has disappeared. On cultures two months old the film can be observed as a thin fatty-looking layer over the surface of the liquid. It is semi-transparent and greyish in colour, and resembles somewhat in appearance the bloom seen on certain fruits, such as plums or grapes. The appearance of the film is the same on either beer-wort dextrose, laevulose or saccharose solutions. The films are formed both at room-temperatures and also at 25–30° C.

Under the microscope, numerous gas-bubbles are seen to be included among the Yeast-cells. Most of the cells are round or slightly oval with sharply-marked cell walls, one or



more large vacuoles, and bright refringent granules. Many, however, are elongated into pear-shaped cells, or rods: and small false mycelia, made up of these forms with a few round cells, are plentifully found. (See Fig. 10, *a. b. c.*, from secondary films on beer-wort, saccharose-Mayer, and laevulose-Mayer respectively).

#### FORMS OF CELLS.

The individual cells of this Yeast are of very different shapes and appearance, according to the conditions of growth, age of the cell, and other causes.

In young vigorous cultures the cells are almost entirely ellipsoidal or slightly egg-shaped, a few being round. They are filled with clear colourless protoplasm and are devoid of granules and vacuoles. The cell-wall is not sharply marked off from the protoplasm, and single cells on account of their homogeneity and transparency can easily be overlooked. This form is the actively growing form, and produces buds rapidly. Sometimes these buds are detached when still very small, this occurring principally in cultures in liquids; they are similar in appearance to the larger cells, but on account of their very small size look like small *Torula*-forms. The usual size of the cell at the time of budding is  $5-7\ \mu$  in length,  $4\ \mu$  in breadth. In old cultures this form of cell is rare.

When the cells just described have finished their active growth and division by budding, vacuoles begin to be formed in their protoplasm, and the cell-wall begins to be more sharply marked off from the cell-contents, the cell also becoming less transparent. Cells in this stage can be found in solutions that are actively fermenting. Later still, the cell-wall is sharply marked off from the rest of the cell, one or more bright refringent granules are formed, and the colour of the cell becomes somewhat brownish. Most of these cells are round, and in size vary from  $3.5\ \mu$ – $8.5\ \mu$ . Some, however, have become elongated to a greater or less extent, and forms pear-shaped, rod-like filamentous, or thread-like are produced. The length of these sometimes is as much as  $17\ \mu$ , while in

breadth the pear-shaped forms are  $8.5\mu$ . Many of these elongated forms are found, placed end to end, forming a false mycelium. These cells with granules are to be found in old cultures, both in solutions and on solid media. They constitute almost the whole of the vegetation in these cases. Among them cells containing spores are usually to be found.

Illustrations of this type of vegetation are found in Fig. 10, *a. b. c.*, taken from secondary films formed on beer-wort, saccharose-Mayer, and laevulose-Mayer solutions respectively.

#### SPORES.

This Yeast is remarkable for the ease with which it can form spores and also for the shape of its spores. They are produced one to four in number in a single cell, the usual number being three or four. They are similar in form to those described by Hansen for *Saccharomyces anomalus*<sup>1</sup>, being shaped like a half-sphere with a horizontally-projecting rim round the edge of the flat surface. From the resemblance to an ordinary 'Bowler' or 'Billy-cock' hat, they may be termed hat-shaped. In size they average  $3.5\mu$ – $4.5\mu$ .

Cells containing spores are always to be found in old cultures, whether on solid media or in liquids. Spores which have escaped from their mother-cells are also abundantly found: those from the same mother-cell usually remain attached by their thickened rims to one another, and show the usual tetrad arrangement.

In order to obtain abundant supplies of spores the following method was adopted:—A few drops of a vigorous twenty-four hours' old culture in beer-wort at  $28^{\circ}\text{C}$ . were poured over sterilized pieces of porous biscuit-porcelain filter-plate. These were allowed to remain at  $25^{\circ}\text{C}$ . for forty-eight hours in a moist chamber, and on examination of the growth formed on the plate numerous spores were found. Abundant spore production takes place at any temperature between  $18^{\circ}\text{C}$ . and  $28^{\circ}\text{C}$ . Blocks of gypsum were tried instead of the porous filter-plate, but the latter gave the better results.

<sup>1</sup> Hansen (1).

The early stages of spore-formation have not been observed in detail, but there is no doubt that they are formed by repeated bipartition of the protoplasm in two successive planes, as in ordinary Yeasts.

#### GERMINATION.

For some time attempts to observe spores in process of germination were unsuccessful, apparently owing to my having used spores not yet fully ripened. Eventually success was attained by using spores developed on pieces of filter-plate, the growth on which had been allowed to thoroughly dry. Cells from the dried layer were well shaken-up in a tube of sterile beer-wort, in order to separate the individual cells completely, and then the tube was heated for five minutes at 55° C. By this procedure the vegetative cells were killed, and on making hanging drops of beer-wort gelatine the germination of the spores could be observed, without being obscured by the rapid growth of vegetative cells which would have taken place under ordinary circumstances.

Observations of the germination of several spores show that the process can be generally stated as follows: the spore begins to swell in about twenty-four hours after sowing at 18° C. The time that elapses before swelling begins is, however, very variable. During the swelling the spore becomes much more transparent than before. It swells until its diameter is about double its original size, viz. from  $3.5\mu$  to  $7\mu$ . A bud is then developed at some point on its surface, the position not being constant (Fig. 11). While this bud increases in size and attains the appearance of an active cell of this Yeast, one or more buds are developed at other points of the spore. When the buds attain the size of an ordinary cell, viz. about  $5-7\mu$ , they in their turn produce buds, and thus a small colony is formed, the individual cells of which separate as the gelatine immediately surrounding them becomes liquefied.

The fate of the thickened rim of the spore has not been



clearly made out, but during the swelling of the spore it becomes much less noticeable. Whether it is a fold which stretches out as the wall extends, or a solid rim the substance of which swells and is used up, could not be determined. The buds are developed, not only on the rounded surface of the spore, but also on the flattened base, as seen in Fig. 11, so that the spore eventually loses to a great extent its hat-like shape.

It may be noticed as a point of interest that there is no preliminary mycelium nor any structure analogous to a pro-mycelium developed during germination, typical Yeast-cells being formed at the first budding.

#### PHYSIOLOGY.

*Temperature limits.* The optimum temperature of growth, as judged by the amount of development in streak-cultures on beer-wort agar-agar, is 28° C. Growth is possible at all temperatures between 15° C. and 32° C. At 10° C. it is very slow, while exposure for five minutes to a temperature of 55° C. kills all, or nearly all the vegetative cells. Exposure for the same period to a temperature of 50° C., however, leaves many cells still living.

*Aerobism.* Complete absence of free oxygen prevents growth, or at any rate prevents its initiation, but the presence of mere traces of that gas will allow growth to continue. Thus, if a flask of beer-wort infected with the Yeast be exhausted by the filter-pump for two hours, or even when the filter-pump is allowed to act continuously, the characteristic film is slowly formed on the surface of the liquid. If a series of plate-cultures be made, using beer-wort gelatine as the medium, and placed under the same conditions, colonies of the usual form are developed. This is probably owing to the impossibility of removing all the air by such means, because if the above experiments are performed in the presence of a solution of pyrogallie acid in caustic soda, in neither case is there a development of the Yeast.

Similar results are obtained if the inoculated flask is placed

in an atmosphere of hydrogen instead of *in vacuo*, the presence of pyrogalllic acid and potash causing growth to cease, while in its absence growth takes place as usual.

These experiments seem to prove that the growth of the organism depends on the presence of free oxygen, since when the last traces of the free gas are absorbed by the alkaline solution of pyrogalllic acid, growth is inhibited.

Other experiments bearing on the same point were made, using fermentation-tubes<sup>1</sup>. These were filled with sterilized beer-wort and infected with the Yeast. It was found that when active fermentation was in progress, that gas frequently accumulated in the vertical closed tube. At first this was taken as indicating the anaerobic power of the Yeast, but this fact being at variance with the results of the experiments mentioned above, led to a repetition of the experiment. The same results were obtained when active fermentation set in, but it was noticed that the beer-wort in the vertical closed tube, instead of becoming cloudy, as one would expect if the Yeast were anaerobic and therefore were developing in that portion of the liquid, remained quite clear. The fermentation-tube was allowed to stand until active fermentation had ceased, and then it was noticed that the gas in the vertical closed tube was gradually absorbed again, until not more than one-twentieth part of the original volume remained. This was not absorbed, although the tube was allowed to stand for four weeks. The experiment was again repeated, and exactly the same results obtained. The explanation seems to be that at the beginning of active fermentation the evolution of carbon dioxide is so rapid that a portion of it strikes against the curved surface and rises by accident into the closed tube, and this is not absorbed until active fermentation has ceased, on account of the continuous evolution of the gas going on during that period. When this evolution stops, however, the liquid, if not fully saturated with the gas, gradually absorbs that portion contained in the tube.

*Liquefaction of gelatine.* The Yeast is capable of liquefying

<sup>1</sup> See Theobald Smith (23).

gelatine, but usually only to a slight extent. The amount of liquefaction that takes place varies considerably. During the autumn of 1899 apparently contradictory results were obtained from observations of the behaviour of plate-cultures of beer-wort gelatine and streak-cultures.

On some plates the gelatine was completely liquefied, while on other plates it remained quite solid: similarly also with streak-cultures. In some cases the gelatine in the neighbourhood of one or two colonies would be liquefied, while on the rest of the plate the gelatine remained solid. Thinking that these results might point to the presence of bacterial infection or of more than one variety of Yeast, the matter was tested by repeating the plate cultures, with the same results. Furthermore, hanging drop cultures from a single colony on a plate on which the gelatine had remained unliquefied were made, using beer-wort gelatine as the medium, the gelatine being in the proportion of ten per cent. The drops were kept under observation under the microscope, and the state of the gelatine in the neighbourhood of the Yeast-cells observed. It remained unliquefied until a colony of cells was produced, and then, in every case observed, it was eventually found to be slowly liquefied in the immediate neighbourhood of the cells, but the liquefaction did not in every case extend to the rest of the gelatine. Also the size of the colony, when the first appearances of liquefaction were observed, varied considerably. In drops four days old sometimes a colony was produced as large as a pin's head, quite dry and glistening white in appearance, and standing out above the surface of the gelatine. In such cases the gelatine was liquefied only in the immediate neighbourhood of the cells imbedded in the gelatine. In other cases the whole drop would be liquefied, and the Yeast would form a greyish-white film over the surface. These facts negative the idea of bacterial infection, since no Bacteria could be observed in the drops, and also the idea of a mixture of Yeasts of different properties, since cases in which drops were made from the same colony show the different results.



All the above experiments were carried on at the room-temperature, which varied from  $15^{\circ}$  C. to  $21^{\circ}$  C. on different days. It was noticed that the higher the temperature, the greater the tendency to complete liquefaction as a rule, although all colonies do not show the same energy. That the temperature itself is not solely responsible for the liquefaction is shown by the fact that when the temperature falls the gelatine does not solidify again, as would be the case if the higher temperature had caused it to melt. Hanging drops in a room, the temperature of which remains fairly constantly as low as  $15^{\circ}$  C., produced the glistening white dry colonies almost invariably, the gelatine being liquefied merely in the immediate neighbourhood of the cells imbedded in it: while hanging drop cultures in a room, the temperature of which was usually three or four degrees higher, showed much more variable results, complete liquefaction often taking place. Plate-cultures, generally speaking, show the same points. In warm weather they were often liquefied, and the usual form of colony was the film-like irregular form, while in colder weather liquefaction was comparatively rare, and the colonies were usually of the glistening white dry form.

The probable state of affairs seems then to be that the Yeast has a feebly developed power to liquefy gelatine, but that its power varies considerably according to the temperature, being much greater at about  $20^{\circ}$  C. than at  $15^{\circ}$  C.

These facts suggest that caution must be employed in deciding that a Yeast is capable of liquefying gelatine, since various circumstances—moisture, temperature, the state of the gelatine, crowding, and vigour of the cells, &c.—may continue to affect the question, just as in the case of the shapes, &c. of the colonies mentioned on p. 219, which in fact depend on the same properties.

#### FERMENTATION.

The capability of this yeast to induce alcoholic fermentation has been tested for the following carbohydrates:—xylose, mannite, gum acacia, dextrin, lactose, maltose, soluble

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starch, dextrose, saccharose, and laevulose. The solutions of each used, with the exception of soluble starch and laevulose, were 10 per cent. of the substance, made up with Yeast extract. Also with the exception of the first three substances mentioned, solutions of 15 per cent. of the carbohydrate made up with Mayer's solution were made, the constitution of each thus being:—

15 grams	carbohydrate.
1 gram	ammonium tartrate.
·5 „	potassium phosphate.
·25 „	magnesium sulphate.
·05 „	calcium phosphate.
100 c.c.	distilled water.

The action of the Yeast on beer-wort was also determined. The results showed that it can ferment saccharose (presumably after inverting it), dextrose, laevulose, and beer-wort, but cannot ferment xylose, dextrine, acacia gum, mannite, lactose, or soluble starch.

With reference to the fermentation of maltose, a few small gas-bubbles are found at the edges of the liquid, when film-formation has taken place. They are not numerous and may be due merely to air enclosed by the film during its formation. If they are due to fermentation, then the fermentation that takes place is very feeble.

A film is formed on each of the above solutions, but in the cases of xylose, gum acacia, and mannite, the growth observed may have been due to the presence of Yeast extract and not to the carbohydrate.

In all the other cases, however, growth was due to the carbohydrate, since cultivations on the substance dissolved in Mayer's solution produced films as well as those on the substance made up with Yeast extract.

Fermentation is of a vigorous character with each of the substances that the Yeast is able to ferment, that of beer-wort, however, not being as vigorous as the others. It started in from two to three days after infection at 28° C. and con-

tinued actively for several days. In the case of dextrose-Mayer solution, of which 250 c.c. were used in an Erlenmeyer flask of 500 c.c. capacity, the evolution of gas continued for two and a half weeks after fermentation had started, and saccharose and laevulose fermentations continued for about the same length of time. Beer-wort fermentations did not last quite so long, or in other words were completed more rapidly. At room-temperature, fermentation proceeded more slowly.

The characters of the vegetation on the different media used were similar, and the typical forms of cells found have been dealt with in the morphological portion of this paper.

#### PRODUCTS OF FERMENTATION.

*Gases.* The gas, collected in the closed vertical tube of the fermentation-tubes mentioned above when dealing with the aerobism of this Yeast, was analyzed. Nearly the whole of it was absorbed by caustic potash solution, thus showing that the bulk of it consisted of carbon dioxide. After the gas had been exposed to the action of potash for some minutes, pyrogallic acid was added, and a further portion was absorbed, this indicating the presence of oxygen. A small quantity of gas still remained unabsorbed and was presumably hydrogen or nitrogen. Stated roughly, the quantities of each present would be in every 100 parts 95 per cent. carbon dioxide, 1 per cent. oxygen, and 4 per cent. nitrogen, which makes it almost certain that the undissolved residue after absorption with potash is air which found its way in during the fermentation, probably from the air entangled among the cells of the primary films.

*Ethyl alcohol.* Fermented solutions of saccharose, dextrose, and laevulose were subjected to distillation and the iodoform test applied to the distillation. Ethyl alcohol was found to be present in each case. The amount present in the distillate, as estimated by an alcoholometer, was as much as 5 per cent. in some cases.

*Higher alcohols* are formed during the course of fermentation



in small quantities, among which amyl and butyl alcohols are predominant.

*Organic acids.* The fermented liquids show a well-defined acid reaction to litmus. Acetic, butyric, and succinic acids have been found.

*Fruit ethers.* One of the most conspicuous points in connexion with the fermentation brought about by this Yeast is the strong odour of fruit ethers that is developed after active fermentation has been in progress for some time. The exact odour varies, but that of ethyl acetate in some cases, and that of amyl acetate in other cases, are predominant. The odour in beer-wort fermentations is not as strong nor as well defined as in the cases of dextrose, saccharose, and laevulose.

#### HISTORY AND BIBLIOGRAPHY.

In 1891 Hansen (1) published a paper, *Sur la germination des spores chez les Saccharomycètes*, in which he gave a short description of a new species, belonging to that genus, which he named *S. anomalus*. The organism was characterized by an extremely quick film-formation on beer-wort, the film appearing at the beginning of fermentation, by the production of a strong odour of 'fruit-ethers' during the course of fermentation, and by the resemblance of the organism, when viewed under the microscope, to certain *Torula*-forms. During fermentation a strong odour of 'fruit-ethers' is produced. It was capable of forming spores under similar conditions to those required by other species of *Saccharomyces*, and they were also to be found plentifully in old cultures.

They differed from the spores of all other species of *Saccharomyces* in their shape: they were hemispherical, with one surface flattened, and they had a projecting rim round the edge of the flattened surface. Hansen called them 'hat-shaped,' and pointed out that they were identical in form with those of *Endomyces decipiens* (18). On germination they gave rise to no pro-mycelium, but, instead, produced buds similar to those developed by ordinary vegetative cells. The paper concludes with a short reference to the possibility of

genetic relationship between this new species and *Endomyces decipiens*.

In a paper published in 1892 Lindner (2) mentions a Yeast with hat-shaped spores. Jörgensen (3) also, in his book *Die Mikroorganismen der Gärungsindustrie*, gives a description of *Saccharomyces anomalus*, and mentions that other observers had found Yeasts with hat-shaped spores, which, apparently, were very closely related to the form described by Hansen, if not actually identical with it.

Ludwig (4) also describes the species. Fischer and Brebeck (5) have investigated an organism, which they have named *Endoblastoderma pulverulentum*, and which they state to be identical with the form *Mycoderma cerevisiae* (var. *pulverulenta*), Beyerinck. It was obtained from the lager-bier of a Rotterdam brewery. They distinguished it from the other species of their genus *Endoblastoderma* by its hat-shaped spores and by the pleasant fruit-like odour produced during fermentation. On beer-wort at 27° C., a white floury film was formed during the second day after infection. On beer a yellowish-white, thick wrinkled film appeared in the course of the first week.

Microscopically examined in young cultures, the cells were mostly round or egg-shaped, and strongly refractive. In older cultures the cells were of very various sizes, and occasionally false mycelia were found. The cells of the films were able to resist a temperature of 80–85° C. when exposed to it for ten minutes. Exposure, however, to a temperature of 60° C. for half an hour sufficed to kill all the cells. The colonies produced on plate-cultures reached a size of 5 m.m. in diameter and lay above the surface of the gelatine. In form they were flattened domes, circular in outline, white, dry, and pitted. They liquefied the gelatine substratum towards the end of the second week. Streak cultures produced complete liquefaction in seven weeks. Before liquefaction the streak-cultures showed a thick white layer, whose upper surface appeared as if it had been strewn with flour. The spores were hat-shaped and were formed usually in threes. The organism fermented beer-wort, dextrose, lae-

vulose, maltose, and saccharose ; the fermentations, except in the case of laevulose, were very vigorous. Their genus *Endoblastoderma* was based on the property of endogenous cell-formation by its members, and they claimed that this manner of formation had been observed by them in the case of the organism just described.

Briefly stated, the method of endogenous cell-formation was as follows:—in a young cell a refractive particle made its appearance, and increasing in size made its way bodily through the cell-wall: it then developed in exactly the same manner as a bud produced in the ordinary manner by constriction from the mother-cell. The authors pointed out the great similarity of their organism to Hansen's *Saccharomyces anomalus*, and laid stress on the fact that the exact relationship could only be determined by testing the power of the latter to form cells endogenously in the manner just described. Klöcker (6) undertook this work, and failed to discover in *S. anomalus* any trace of endogenous cell-formation apart from the ordinary spore-formation. He noticed, however, the appearances on which Fischer and Brebeck based their view that endogenous cell-formation took place, and found that they were due to ordinary budding, the buds in some cells being produced in a direction more or less vertical to the cover-slip. On growing, these buds shifted their position in such a way that they appeared to move from the inside of the mother-cell to its exterior. Similar results were also obtained by him, when a variety of *Mycoderma* was investigated. There appears thus to be no ground for Fischer and Brebeck's construction of the new genus, *Endoblastoderma*. Klöcker stated in his paper that there could be no doubt that *S. anomalus* and *E. pulverulentum* are identical, from the description given of the latter.

Nielson (7) has investigated the effects of temperature on spore-production by *S. anomalus*. At temperatures above 33° C. no spores were formed. At 30° C. they made their first appearance in seventeen to nineteen hours ; at 28° C. in seventeen and a half to nineteen hours ; and at 25° C. in



eighteen to twenty hours. At  $7\frac{1}{2}$ – $6^{\circ}$  C. thirteen to fourteen days were required before signs of spore-formation appeared, and at  $2\frac{1}{2}$ – $3^{\circ}$  C. no spores were produced.

In Hansen's work (8) on the duration of life of various Yeasts under different conditions, it is stated that *S. anomalus* was able to live for more than eighty days, when spread in thin layers on a platinum wire needle. Most Yeasts died in five to twenty days.

Wehmer (9), in his studies on the capability of various Fungi to liquefy gelatine, found that *S. anomalus* did not liquefy 10 per cent. beer-wort gelatine. At the same time he draws attention to the fact that the form described by Fischer and Brebeck (5) under the name *Endoblastoderma pulverulentum* peptonized the gelatine.

Von Schukow (10) found that *S. anomalus* produced very little fermentation in unhopped beer-wort at  $20$ – $22^{\circ}$  Réaumur; the fermentation was only about a quarter as vigorous as with many beer and wild Yeasts. He suggested that this was due to the fact that only the dextrose contained in the wort was capable of being fermented by the species.

*S. anomalus* has been met with in saké fermentations. Its presence has been noted by Klöcker and Schiönning (11) and by Shieweck (12). The latter observer thinks that in conjunction with other Yeasts it plays an important part in the fermentation. This is very probable, since a strong odour of pine-apples is developed during the course of fermentation in the saké. This odour may be due to the formation of ethyl butyrate, and it has been shown above that the variety which I have investigated produces butyric acid. Yabé (13) has found a *Mycoderma* yeast growing on saké rice. Two other papers on *S. anomalus*, one by Steuber (14) and the other by Kujawski (15), have been recently published, but I have not had the opportunity of seeing them.

From a comparison of the various statements quoted above, of the different observers who have investigated the characters of *S. anomalus*—and we may accept Fischer and Brebeck's *Endoblastoderma pulverulentum* as a form of this species—it

appears that the distinguishing features of the species are the *Mycoderma*-like habit and the hat-shaped spores. Apart from these there seems to be considerable variability in the characters which have been noted. For instance, Fischer and Brebeck found that their organism ferments beer-wort, dextrose, saccharose, and maltose actively, while the fermentation of laevulose is less vigorous. Von Schukow found that his organism was able to produce only a comparatively slight fermentation in beer-wort, and accounts for it by supposing that only the dextrose is fermented. Certainly if the Yeast had been able to ferment maltose, a much more vigorous fermentation of beer-wort would have been expected.

The variety that I have examined ferments dextrose, laevulose, and saccharose actively, beer-wort less actively, and maltose in a very slight degree, if at all.

Different results, also, were obtained in connexion with the question of the liquefaction of gelatine. Fischer and Brebeck's organism liquefied gelatine completely; Wehmer's produced no liquefaction; while that described by me showed the variable behaviour that has been fully dealt with above.

Fischer and Brebeck found that exposure to a temperature of 80° C. for ten minutes did not suffice to kill the cells of their Yeast. Exposure to a temperature of 55° C. for five minutes was sufficient to kill the vegetative cells of my Yeast.

With regard to the number of spores formed by a single cell, Fischer and Brebeck found that three was the usual number. In the cases examined by me, although while the spores still remained within the mother-cell wall the number appeared to be three, yet probably four were present in most cases, the fourth being obscured by the positions of the other three: for when the spores had escaped from the mother-cell they were found chiefly in fours, grouped in tetrad arrangement, the fourth spore only coming into view as the group revolved in the liquid in which they were mounted.

The lack of detailed descriptions of the Fungi with hat-shaped spores<sup>1</sup> that have been found at various times renders

<sup>1</sup> *Ascoidea rubescens* (Bref.) also has similar spores. See Brefeld (18).

a complete comparison impossible, and conclusions as to the identity or relationship of the Yeasts can only be surmised, though they are extremely probable. It does seem probable, however, that there are differences between some of the forms, at least as great as there are between the different varieties included in the species, *S. cerevisiae* or in *S. Pastorianus*. It is interesting to note the wide distribution of these forms, some having been found in Japan, others in various parts of Europe, while quite possibly the form described in this paper is a West Indian form, the ginger on which it was found having come from Jamaica. Their wide distribution alone would lead one to expect considerable variety in their characters. The most satisfactory way of grouping them seems to be the inclusion of each form in the species *S. anomalus*; this species to have as its distinguishing characters the *Mycoderma*-like habit and the hat-shaped spores; and the subdivision of this species into varieties according to the behaviour of the Yeast in such points as the power of fermenting the various carbohydrates, the usual number of spores produced, the nature and rate of production of primary films, the occurrence and appearance of secondary films, the power of liquefaction of gelatine, the production of fruit-ethers<sup>1</sup>, &c. The species would then have the same significance and would show a similar amount of diversity among its members, and yet be just as well defined as the better known species of *Saccharomyces*, such as *S. cerevisiae* and *S. Pastorianus*.

#### ON THE QUESTION OF RELATIONSHIP BETWEEN *S. ANOMALUS* AND *ENDOMYCES DECIPIENS*.

In the paper by Hansen (1) quoted above it will be noticed that he draws attention to the possibility of genetic connexion between *S. anomalus* and *Endomyces decipiens*, on account of the similarity in shape of the spores of the two species, bearing in mind at the same time the position held by the latter as one of the simplest types of the group Exoasci and

<sup>1</sup> Beyerinck (21) describes other species of Yeast which produce fragrant fruit-ethers, *S. fragrans* and *S. acetaethylicus*, apparently quite distinct from *S. anomalus*.



the generally accepted classification of *Saccharomyces* with the Ascomycetes. In connexion with this question it is of interest to note the conclusion arrived at by Hansen (16) on the possible genetic relationship between *S. Ludwigii*, which in many respects shows a close connexion with *S. anomalous*, and another species of *Endomyces*, viz. *E. Magnusii*. These forms were discovered by Ludwig (17) in the 'Schleimfluss' of an oak, and this observer speaks with great reservation on the important question of their relationship. Brefeld (18) further investigated the form *Endomyces Magnusii*, and Hansen (19) has published a full account of *S. Ludwigii*. Both these observers agree in the conclusion that there is no clear evidence of undoubted genetic relationship between the two forms. I may also point out that recent attempts to connect the genus *Saccharomyces* with mycelial Fungi have broken down under the experimental criticism of Klöcker and Schiöningg (22).

Returning to the consideration of the other species, Brefeld (18) has given a description of *E. decipiens* in his account of the Exoasci, and also numerous figures of the species. The form is found as a parasite on the lamellae of *Agaricus melleus*, and consists of a branched mycelium, composed of elongated cells placed end on end. On the older parts of the mycelium asci are borne. They arise as short side-branches, which swell up and produce in their interior four hat-shaped spores. These spores are capable of germination immediately after ripening. In nutrient solutions they swell up, lose their original form, and from one or more points short tubes are developed which quickly grow to a branched mycelium. After two or three days the mycelium begins to break up into oidia. In the course of culture mycelia are produced, which are nothing more than long chains of oidia. Later, also, chlamydospores are produced singly on short side branches of a mycelium. They consist of a single large cell, with a yellow and thickened cell-wall, and contents rich in fat. Brefeld points out that they are morphologically equivalent to the oidia. On culture-solutions a thick white

film is formed, which originally consists entirely of oidia, but later chlamydospores appear and give to the film a yellowish colour.

On purely morphological grounds it will be noticed that there are certain resemblances between the two forms, but with reference to the mycelial formations, it would appear that here there is a distinct difference. Fig. 12, however, taken from the edge of a colony of *S. anomalus*, shows that there is a great tendency in this organism to produce a mycelium on a solid and dry medium. The asci in the two cases present considerable differences however. In *S. anomalus*, as far as we know, an ordinary vegetative cell becomes developed into an ascus under suitable conditions, while in *E. decipiens* the ascus is developed from a side-branch of the mycelium, i.e. in a somewhat definite position. This distinction, however, must perhaps not be pressed too much until definite knowledge is obtainable as to the power of every vegetative cell of the Yeast to form ascospores, and it is conceivable that it points merely to a slightly more specialized condition in the case of *E. decipiens*. The ascospores of both forms resemble one another completely in shape. Each ascus in *E. decipiens* typically produced four spores, while four is the most usual number in the form of *S. anomalus* which I have described. The germination of these spores, however, differs in a marked degree owing to the formation of a pro-mycelium by *E. decipiens*. The question may be raised whether this difference is as important as it appears at first sight. Hansen (1) has shown that the spores of *S. Ludwigii*, a species which is nearly related to *S. anomalus*, produce a sort of pro-mycelium on germination, while Reess (20) pointed out that the spores of *Taphrina Pruni* (Tul.) germinate in the same way as ordinary *Saccharomyces* spores, i.e. by budding. Brefeld (18) has shown the same for *Taphrina rhizophora* (Johans). The genus *Taphrina* is usually regarded as related to *Endomyces*.

From these examples, then, it does not follow that the production and non-production of a pro-mycelium are facts

which preclude the possibility of close relationship between two forms.

It might be argued that the formation of oidia by *E. decipiens* has a parallel in the case of *S. anomalus*, in the production of the rod-like cells, mentioned above as occurring in old cultures, and some of the forms of oidia figured in Brefeld's work are not dissimilar in appearance from the rod-like cells of *S. anomalus*, the 'mycelioid' forms of which, moreover, might be consistently regarded as series of oidia, if it were not for the fact that they are developed by budding. Nevertheless, the morphological equivalent for *S. anomalus* of the chlamydospores of *E. decipiens* has not yet been definitely determined, and even if it seems probable that the brownish-coloured large rounded cells, with well-defined cell-wall and fat globules, of the latter are practically chlamydospores, we can scarcely go so far as to argue their morphological identity. However, Hansen (8) has mentioned the power of resistance by the cells of *S. anomalus* to unfavourable surroundings, and it has been seen that in old cultures, for instance, the cells acquire the characters just described. In the case of the Yeast, any cell seems to be able to acquire the characters of a chlamydospore in a certain sense, but in *E. decipiens* these spores are usually developed in more or less definite positions, that is to say at the ends of side branches. Attention, however, must be drawn to the fact that Brefeld figures (Plate I, Fig. 27, 2, in his work quoted above) a chain of oidia, apparently not developed in any special position, which were acquiring the characters of chlamydospores. Hence the ordinary oidial cell of *E. decipiens* can be looked upon morphologically as a chlamydospore, thus corresponding in this capacity to the ordinary vegetative cells of the Yeast.

To carry the resemblance between the two species still further, it might be insisted that both form white films on the surfaces of nutrient solutions, and the older films of both show the presence of chlamydospores or cells that are equivalent to chlamydospores.



The property of inducing fermentation possessed by *S. anomalus* is absent in the case of *E. decipiens*. Perhaps this difference in behaviour need not be insisted upon as indicating a wide gap in the relations of these forms, for the behaviour of the various species of *Saccharomyces* with regard to different sugars, and even, as we have seen, the behaviour of different varieties of *S. anomalus* also, is so variable that it cannot be held to affect relationships higher than varieties. Still we find such films in many Fungi, and so cannot push the argument.

From this comparison of the two species, while there is no ground for supposing that *S. anomalus* is in any way a form of *E. decipiens* that has taken on a more or less permanent Yeast-like habit, yet there are indications of a relationship between the two species, and possibly a case could be made out for the view that the *Saccharomycetes* had their origin from the Exoasci, with such forms as *S. anomalus* and *E. decipiens* as connecting links, *Ascoidea rubescens* also, with considerably more divergent characters, being taken into the purview. As a matter of fact, however, no proof of the direct connexion between this or any true Yeast and a typically mycelial fungus has yet been brought forward. See Klöcker and Schöning (22).

In conclusion I should like to take this opportunity of stating that this work has been carried on in the Cambridge University Botanical Laboratory, by permission of Professor Marshall Ward, to whom my thanks are also due for his unfailing help and advice.

**Note.** Since the above was in print I have been able to see a copy of Steuber's paper (14) in the *Centralblatt für Bakteriologie, &c.*, Abth. II, Bd. VI, No. 7. The author describes four varieties of *S. anomalus*, which differ considerably with regard to the forms of the colonies on plate-cultures, temperature-limits for vegetative growth and spore-production, liquefaction of gelatine, and behaviour with various sugars. The 'variety I' approaches most nearly the form described above. It ferments dextrose, laevulose, and saccharose completely, but

does not ferment maltose, lactose, and galactose, being able, however, to grow on solutions of the latter sugars. There is an abundant formation of ethyl acetate and acetic acid, together with butyric acid; but the author makes no mention of amyl acetate, which is produced abundantly by the variety which I have investigated. It liquefies gelatine comparatively rapidly, and the maximum temperature for film-formation is rather higher than that for my variety, viz.  $37^{\circ}$ – $40^{\circ}$ C. Judging from the description, the forms of the colonies on 10% beer-wort gelatine resemble those shown in Fig. 2.

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## EXPLANATION OF FIGURES IN PLATE XIII.

Illustrating Mr. Barker's paper on a *Mycoderma* Yeast.

Fig. 1. Photograph of colonies on a plate of laevulose-Mayer gelatine (nat. size). Darker colonies becoming moist through absorption of liquefied gelatine. Two months old. At ordinary temperatures.

Fig. 2. Photograph of colonies on a plate of beer-wort gelatine (nat. size). Two months old. At ordinary temperatures.

Fig. 3. Plate-colonies on beer-wort gelatine at ordinary temperatures. The gelatine had partially liquefied. A, B, C, D, E, F, successive stages as shown on a seven days' plate.  $\times 2$ .

Fig. 4. Colony on beer-wort gelatine plate. Gelatine solid. One month old. At ordinary temperatures.  $\times 4$ .

Fig. 5. Beer-wort gelatine plate-colony at ordinary temperatures, showing mycelium formation. Two months old.  $\times 4$ .

Fig. 6. Colonies on saccharose-gelatine plate at ordinary temperatures. Three months old. A. Dot-like colony. B. Colony with partial mycelial development. C. Colony with well-developed mycelium.  $\times 6$ .

Fig. 7. Plate-colony on laevulose-Mayer gelatine at ordinary temperatures. One month old. A. As seen from above. B. Side view.  $\times 6$ .

Fig. 8. Fatty-looking plate-colony on laevulose-Mayer gelatine at ordinary temperatures. One month old.  $\times 4$ .

Fig. 9. Successive stages of cell-formation by budding from a single cell. A, at 1.45 p.m. B, at 2.55 p.m. C, at 4.20 p.m. D, at 5.30 p.m. E, at 7.30 p.m. From hanging-drop culture in beer-wort gelatine. Gelatine partially liquefied, thus accounting for changes in position of the cells. At 18.5°–19° C.  $\times 1000$ .

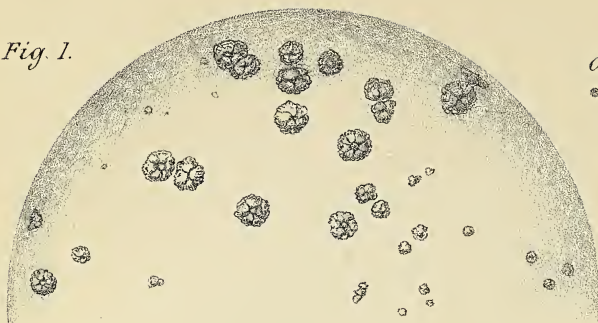
Fig. 10. Vegetation of secondary films (veils). A, on beer-wort. B, on saccharose-Mayer solution. C, on laevulose-Mayer solution.  $\times 480$ .

Fig. 11. Stages in the germination of spores at 15° C. Group of four spores. A, at 3 p.m. B, at 9.45 a.m., three days later. C, at 11.15 a.m. D, at 2 p.m. E, at 4.35 p.m. F, at 9.30 p.m. Single spore: G, at 3 p.m. H, at 9.30 a.m., four days later. I, at 1.15 p.m.  $\times 1000$ .

Fig. 12. Portion of mycelium developed at edge of plate-colony on beer-wort gelatine (see Fig. 5). A. Terminal filament. B. Older branched portion.  $\times 480$ .

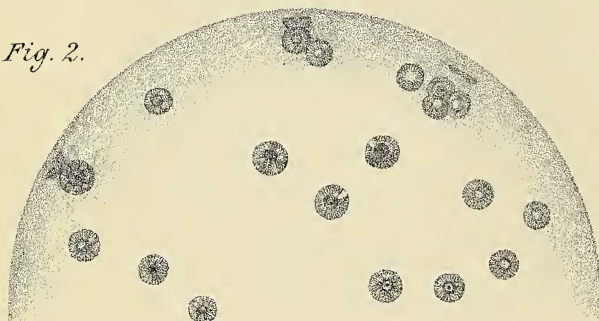


*Fig. 1.*

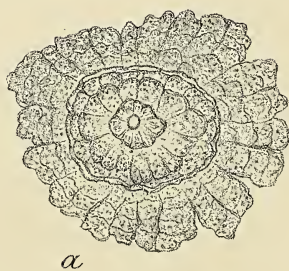
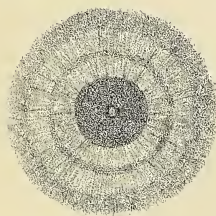


*Fig. 3.*

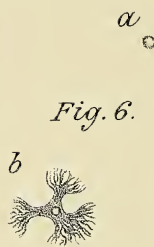
*Fig. 2.*



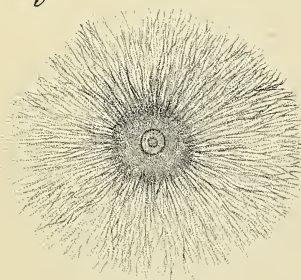
*Fig. 4.*



*Fig. 6.*



*Fig. 5.*



*Fig. 7.*



*Fig. 8.*



Barker del.

BARKER. — A FRAGRANT "MYCODERMA" YEAST.



Fig. 9.

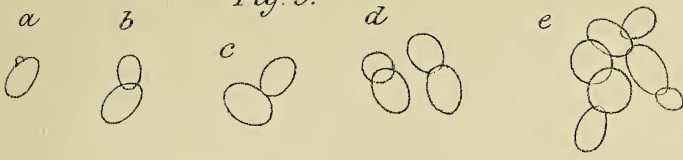


Fig. 10.

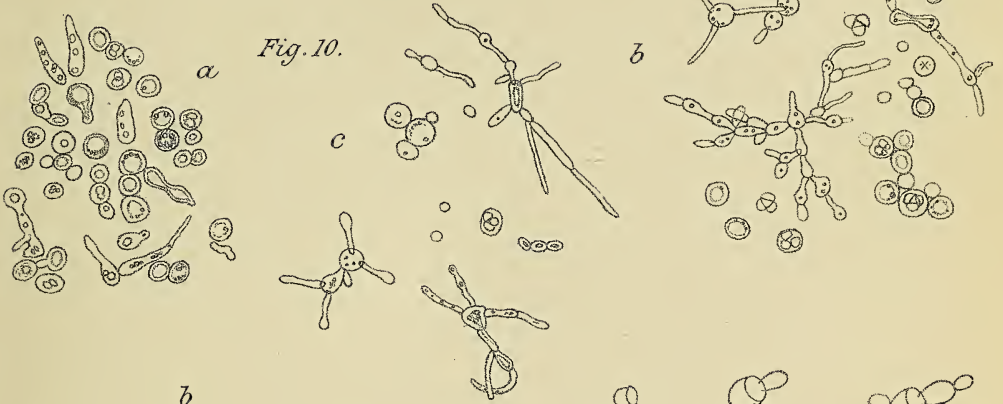


Fig. 11.

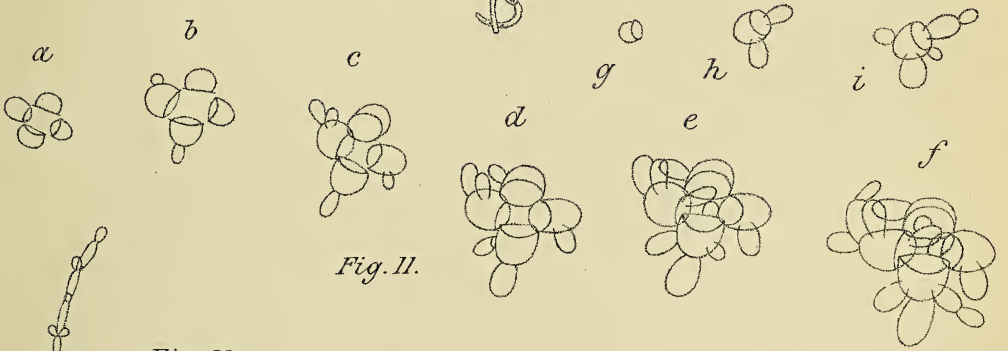
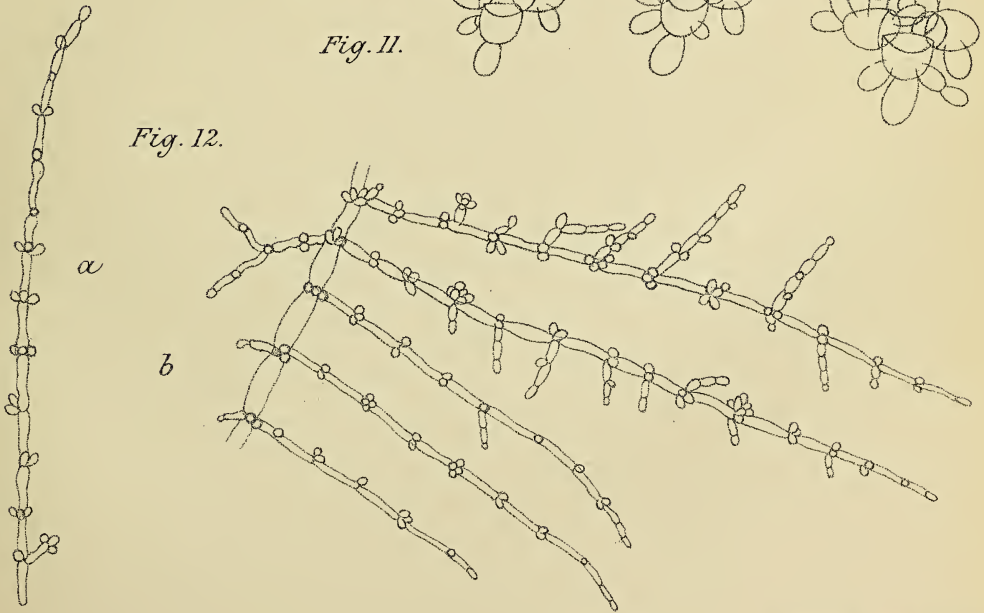


Fig. 12.





# On the Biology of *Poronia punctata* (L.).

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With Plates XIV and XV.



THE genus *Poronia* was founded by Willdenow<sup>1</sup> in 1787, and up to 1882 it is given by Saccardo<sup>2</sup> as containing seven species. In Lindau's<sup>3</sup> classification eleven species are described.

The species here concerned was first described by Linnaeus.

Up to the present time but few investigators appear to have devoted their attention to *Poronia*. With the exception of a brief description of the external features of *Poronia punctata*, with excellent figures of the stromata, asci, and spores by Tulasne<sup>4</sup>, and a similar description, with an account of the cultivation of its spores and conidia-bearing stromata by Brefeld<sup>5</sup>, a mere passing reference is made to this Fungus in various contributions to our knowledge of the Ascomycetes.

<sup>1</sup> Willdenow, Flor. Berol. Prodr., p. 400.

<sup>2</sup> Saccardo, Sylloge Fung., vol. i, p. 348.

<sup>3</sup> See Engler and Prantl, Pflanzenfamilien, vol. i, Abt. i, p. 490.

<sup>4</sup> Tulasne, Carpologia, ii, p. 27, tab. III.

<sup>5</sup> Brefeld, Untersuch. a. d. Gesamtgebiet d. Mykologie, Heft x, p. 261.



It is usually cited as another example of the type of life-history exemplified by *Xylaria*, or as a member of the group Xylarieae, forming characteristic stromata, with one definite type of conidia, followed later by perithecia with asci and ascospores<sup>1</sup>.

A rough examination of the Fungus was sufficient to show that, in common with other members of its group, it offers many very interesting points for investigation; accordingly, at the suggestion of Professor Marshall Ward, to whom my thanks are due for many suggestions throughout the work, I undertook a somewhat detailed study of the species *Poronia punctata*, in November, 1899. For the material on which the work was carried out I am indebted to Dr. Plowright, who collected it in the neighbourhood of King's Lynn, Norfolk.

#### LIFE-HISTORY.

*Poronia punctata* is an Ascomycetous Fungus, growing upon horse-dung, and characterized by its pegtop-shaped stromata with a whitish disk-like upper surface, mounted upon an unbranched stalk, rarely more than 1 cm. in height, and either standing erect above the surface of the substratum, or, as frequently happens, embedded within the substratum, so that only the expanded disk-shaped head is exposed to view (Plate XIV, Figs. 1 and 2).

In the earlier stages of development the upper parts of the stromata are covered with a greyish white powder, the conidia; these are followed by black spots, scattered over the flat surface of the disk. These spots are the ostioles of the perithecia, which are embedded in the uppermost layers of the stromata, an arrangement which led Berkeley<sup>2</sup> to draw attention to the superficial resemblance of these stromata to the antheridiophores of *Marchantia*. When the asci are

<sup>1</sup> E.g. Fuisting, Bot. Ztg., 1867-68; Cornu, Ann. d.Sci. Nat. (Bot.), sér. vi, 1876; De Bary, Comp. Morph. Fungi and Mycet., p. 244; Zopf, Die Pilze, 1890; Von Tavel, Vergl. Morph. d. Pilze, p. 92.

<sup>2</sup> Berkeley, Introd. to Crypt. Bot., 1857, p. 441.

quite ripe they protrude above the surface in minute black pillar-like masses, enclosing numerous ascospores. A longitudinal section of a mature stroma shows the perithecia in various stages of development, embedded in the vegetative portion of the stroma-head. Immature perithecia appear as more or less spherical dark patches at different levels below the surface, mature perithecia as flask-shaped bodies, opening on the surface (Plate XV, Fig. 16), and bounded by a very definite wall. The asci are club-shaped, and enclose eight dark brown ascospores, ellipsoidal in form, and having a lateral slit-like pit in the outer wall (Fig. 33). They measure 22 by 10  $\mu$ .

Among the asci are numerous colourless, long, slender, multicellular paraphyses. The conidia are small colourless pear-shaped bodies, with oil-like drops, and are formed by abstriction from the ends and sides of the terminal hyphae of a stroma. In some cases the stromata do not expand above into a disk-shaped head, but remain columnar in shape, in which case conidia only are formed, and there is no trace of perithecia (cf. Fig. 3).

For the purposes of pure cultures of the Fungus, groups of ascospores were collected from ripe perithecia by inverting the head of a stroma upon a sterile coverglass, on which it deposited numerous spores overnight. These were washed into a tube of sterile water, and thence plate- and hanging-drop cultures were made, in a medium consisting of 10 per cent. gelatine with a decoction of horse-dung.

For similar cultures of conidia, abundant material could be obtained from any young stroma. In this medium both kinds of spores germinated readily, at the ordinary temperatures of the laboratory (15°–20° C.).

The *Ascospores* form a lateral germ-tube, growing out through the slit-like pit of the exosporium; the germ-tubes are broad, with frequent septa, and the cells are filled with abundant vacuolated protoplasm. Lateral branches arise from points just below a septum, and, as a rule, alternately from left to right of the main hypha. Before definite

elongation of the apex of a hypha, a marked constriction of the extreme end is noticeable, forming a kind of bud, and after considerable elongation of the bud-like protuberance a septum forms at the point of constriction (Fig. 13 *a-h*).

After twenty-four hours' growth frequent lateral anastomoses are seen in the young mycelium (Fig. 15 *a, b*), also the exudation of glistening drops—possibly containing a ferment-like substance—from the tips of the hyphae.

The *Conidia* form germ-tubes from either end or from both ends simultaneously; occasionally three or four germ-tubes may arise from one spore (Figs. 11 and 12). The mycelium produced consists of branched and septate hyphae, at first very slender, but gradually becoming stronger, and eventually indistinguishable from the mycelium grown from an ascospore<sup>1</sup>.

If a young mycelium (grown either from a conidium or an ascospore) be transferred to a tube of horse-dung extract in gelatine or agar, in about three days a beautiful stellate growth is seen, consisting of radiating and glistening silky hyphae; after twelve to fourteen days central heaped-up masses become noticeable, and in three weeks' time minute pillar-like structures, standing up from the surface in the manner shown in Fig. 3 *a*. A large number of young stromata of this kind have been cultivated, and from these have grown in great abundance stromata of unusually large size, frequently branched, and having the various forms found in nature, also bearing large numbers of conidia on the upper parts. Thus far, only early stages in the formation of perithecia have been found; further reference will be made to these later.

In Figures 3 and 4, sketches of some of these stromata, made at different stages in their development, are shown. These cultures have brought to notice several interesting points in the biology of the Fungus. From an examination of a very young stage (see Fig. 3 *a*) we find that this consists entirely of vegetative hyphae, densely interwoven, and rising

<sup>1</sup> Early stages in the germination of ascospores and conidia were figured by Tulasne, loc. cit., tab. III.



vertically from the surface of the horizontal mycelium, which has grown upon and within the substratum. This stage is shown under a low-power objective in Fig. 8, in which must also be noted the presence of numerous crystals of calcium oxalate, which have been separated from the substratum (Fig. 9). Similar crystals were also found in hanging-drop cultures about eleven days after infection (Fig. 10).

When the stromata have reached about half an inch in height they begin to expand at the top, and in some cases become considerably branched; in others, and this is the commoner case, the head continues to enlarge gradually into the form of an inverted cone or pegtop, and a flattish disk or cup-shaped surface is formed above, exactly comparable to those grown in nature. It is noticeable, however, that in these artificial cultures larger specimens were obtained than any which formed from the original material on horse-dung. In the former series several stromata measured 2.5, or even 5 to 6 cms. in height, whilst in the latter none grew to more than 1.5 cm.

It was frequently remarked that if, in the transference of the cultures from one medium to another, a stroma was touched or injured in any way, rapid growth at once took place at the point of injury, and new stromata were quickly formed as lateral branches of the older one (cf. Fig. 5). At all points where growth is vigorous can be seen numerous drops of a pinkish or yellowish fluid; this occurs, not only before the formation of a stroma, but also from the disk-like surface of the fully formed stroma, at the time of the formation of perithecia and asci.

As these mature, the liquid drops dry up, and the black spots, indicative of the ripening perithecia, become visible. The copious exudation of liquid is a prelude to the formation of the perithecia and ascospores. The possibility of its having anything to do with the projection of the trichogyne-like hyphae referred to below cannot be denied, but, since no spermatia or fertilization could be discovered, it seems more probable that the process is rather comparable

with the excretion of fluid drops so often seen in Fungi when spores are being initiated, e. g. in *Pilobolus*. The phenomenon seems to partake of the nature of root-pressure in higher plants, occurring as it does at a time when a considerable supply of food material would be required from the substratum; it is at present, however, without any adequate explanation.

The natural substratum being horse-dung, it is not improbable that cellulose, or some product of its fermentation, is utilized by the Fungus; hence the later stages in the cultivation of these stromata were carried out on cotton-wool, thoroughly soaked with an extract of horse-dung, since after several days' culture on gelatine this medium was completely liquefied. An agar medium proved much less suitable than cotton-wool, probably owing to the rapidity with which the former dried up; on the contrary, cultures transferred from agar to cotton-wool at once began to grow vigorously and to send up new stromata.

From the earliest stages these stromata appear to be strongly apogeotropic; after a month or six weeks it was found that growth was more rapid at a temperature ranging from  $10^{\circ}$ – $13^{\circ}$  C. than from  $15^{\circ}$ – $18^{\circ}$ , and again, that increased aëration of the cultures led to increased development, both in respect to the strength of the stromata and to the rate of growth.

With regard to the effect of injury to any part of the Fungus, it may be mentioned that in every case, when any portion of a stroma was cut off, e. g. the apical part of the one to the extreme left of Fig. 4 *e*, for purposes of investigation, the cut surface of the stalk invariably grew out and formed a new stroma, which frequently developed into a specimen as strong as, or even stronger, than the one cut off. (See Fig. 6, *a-d*, which represents the development of the left-hand stroma of Fig. 4 *e*, after the removal of the slightly swollen head.)

# INTERNAL ANATOMY.

For the study of the internal anatomy of *Poronia punctata*, specimens of stromata were chosen, of different ages, and grown both in an unsterilized medium, as found in nature, and in pure culture. These were hardened in various fixing solutions, e.g. Keiser's<sup>1</sup> solution, absolute alcohol, Rath's, Flemming's<sup>2</sup>, and Hermann's<sup>2</sup> solutions: of these the two last gave the most satisfactory results. The sections were stained with iron-alum, and haematoxylin, according to the method given by Heidenhain<sup>3</sup>. Many other methods of staining were tried, such as those employed by Dittrich<sup>4</sup>, Harper<sup>5</sup>, and Gjurasin<sup>6</sup>, but the haematoxylin stain in most cases proved the most convenient as well as the most useful.

As already mentioned, in the very earliest stages no differentiation can be seen in the hyphae composing the young stroma (Fig. 8). As soon, however, as the head begins to expand, there can be seen scattered amongst the hyphae of the middle and upper part of the stalk more deeply stainable hyphae, with swollen clavate ends, apparently filled with food material.

What the true nature and function of these hyphae may be, I am unable to say, but it seems significant, that whilst they are very abundant in the lower central and especially in the marginal portions of still further developed stromata, which show young stages in the formation of perithecia, they are apparently not to be found either in old specimens with ripe perithecia or in mature stromata which have formed conidia only and as yet no perithecia. In this connexion compare Figures 17, 18, 31, which represent sections of specimens grown,

<sup>1</sup> See Dittrich, Zur Entwicklungsgeschichte der Helvellineen. Cohn's Beitr. viii, 1898.

<sup>2</sup> Zimmermann, Microtechnique.

<sup>3</sup> See Dittrich, loc. cit.

<sup>4</sup> Ibid.

<sup>5</sup> Harper, Pringsh. Jahrb. xxix, xxx.

<sup>6</sup> Gjurasin, Ueber die Kerntheilung in den Schläuchen von *Peziza vesiculosa*, Ber. d. Deutsch. Bot. Ges. xi, 1893.



some under natural conditions and others in pure cultures, in captivity. The distribution, described above, of these swollen hyphae seems to indicate that they may possibly have some important connexion with the formation of perithecia, and they may indeed be genetically connected with the hyphae from which these arise. Moreover it is not impossible that at even this early stage in the development of perithecia some fusion of the contents of two different hyphae may have taken place; but it must be acknowledged that, in spite of a careful search over a large number of preparations, no proof of such a union could be found, although, as Fig. 18 *b* shows, the nucleus in the swollen apical cell of the hyphae is of an unusually large size.

These hyphae present a striking resemblance to the deeply stained hyphae figured by Marshall Ward<sup>1</sup> in *Stereum hirsutum*, and also to the very young ascogenous hyphae described by Krabbe<sup>2</sup> in *Cladonia stellata*, though in this latter case they are related to one ascus only and not to a whole apothecium.

Similar early stages in the formation of ascogenous hyphae have been figured by Dittrich<sup>3</sup> in *Mitrula phalloides*. There is also the possibility that these clavate hyphae are members of a system of conducting tissue such as that described by Istvanffi<sup>4</sup> in some species of *Stereum* and *Radulum*.

If sections of a slightly older stroma than that shown in Fig. 17 be examined, it will be seen that from the outermost layers of hyphae numerous conidia have been formed, giving rise to a thick covering of spores over the top and sides of the swollen portion of the head. This reminds us of the behaviour of the stromata of the closely related *Xylaria*<sup>5</sup>, and indeed of many other Ascomycetes, *Nectria*, *Claviceps*, &c., which always produce crops of superficial conidia before the

<sup>1</sup> Marshall Ward, Phil. Trans., vol. clxxxix, 1897.

<sup>2</sup> Krabbe; cf. Von Tavel, Vergleichende Morphologie der Pilze, 1892, Fig. 31.

<sup>3</sup> Dittrich, Cohn's Beiträge, viii, 1898, Figs. 1, 5, 7.

<sup>4</sup> Istvanffi, Bot. Centralbl., 1887; Prings. Jahrb., 1896.

<sup>5</sup> Cf. De Bary, Comp. Morph. Fungi, p. 244.

perithecia, also of Marshall Ward's<sup>1</sup> discovery that the somewhat similarly shaped stromata of *Onygena* produce chlamydospores on the surface before the asci are developed inside.

At different levels below the surface of the stroma of *Poronia* are visible circular knots of hyphae, distinguished from the surrounding hyphae by their smaller diameter and by their staining more readily. Within these knots of hyphae are seen much larger and still more deeply stained portions of a coiled hypha, which is, in fact, the 'Woronin's hypha' described by Fuisting<sup>2</sup> in *Polystigma* as well as in *Xylaria* and other members of this group (e.g. Figs. 16, 20, 21). The width of this hypha in *Poronia punctata* is 4.4  $\mu$ . Further sections showed portions of this hypha passing up from below through the vegetative parts of the stroma into the coil, and in very occasional instances this was seen after it had formed one or two turns in the coil, but before it had become invested by the surrounding knot of hyphae (cf. Figs. 27, 19, *a* and *b*).

After the formation of the coiled row of cells this hyphae grows on beyond the coil towards the surface of the stroma (Figs. 20, 21). This portion, however, is always more slender than the cells forming the coil, and though in several instances it was seen running right up to the surface, reminding one of the trichogyne of *Collema*, *Physcia*, or *Polystigma*, no evidence could be obtained that it functions as such.

Although hundreds of serial preparations were examined, in no case was a section found which showed the hypha through the whole of its course, indeed it was rare to find the portions growing towards the knots shown in the same stroma, whose sections showed several instances of the 'trichogyne' process. This would suggest that the lower portion quickly disappears, or is emptied of its contents after the early stages of the perithecium are completed. In addition to the deeply stained knots of hyphae enclosing the Woronin's

<sup>1</sup> Marshall Ward, *Onygena equina*, Willd., a Horn-destroying Fungus. Phil. Trans., vol. cxc, 1899.

<sup>2</sup> Fuisting, Zur Entwicklungsgeschichte der Pyrenomyceten. Bot. Ztg., 1867-1868.

coil, may be seen numerous other groups of hyphae, of a similar character, but apparently empty and somewhat less closely interwoven. These probably represent one of two things:—(a) very young stages of perithecia before the formation of Woronin's hypha; or, (b) abortive perithecia, which will never come to maturity.

Having regard to the large number of young stages always present in young stromata, compared with the number of mature perithecia scattered over ripe stromata, it seems very probable that very many such coils are abortive, possibly owing to lack of food-material for the formation of such large numbers of perithecia and asci, the competition of the successful perithecia, which attract all the supplies, being too much for them.

On the other hand, the question arises whether the coil always develops from a definite hypha, which grows up from the main body of the stroma, and after coiling up continues its growth on towards the surface, and as a result of this coiling the neighbouring hyphae grow round and invest it—as seems to be the case—or whether the knot of smaller hyphae is sometimes at any rate formed first, and the Woronin's coil developed within this afterwards.

By more active growth of the investing hyphae on the upper side of the Woronin's coil, the young perithecium gradually loses its circular form and becomes pear-shaped. At this stage the coil is still apparent (Fig. 21), but as the upper part of the perithecium becomes more and more enlarged, the coil appears to be broken up into its component cells, and ere long we find a stage in which the body has become quite pear-shaped, though still lying some distance below the surface, and has the narrower upper portion lined with delicate undivided hyphae—the periphyses—whilst at the base is a group of stouter deeply stained hyphae, presumably the remains of the coil, and now representing the young ascogenous hyphae (Fig. 23). By the continued growth of the lateral hyphae of the perithecial wall, possibly aided by the solvent action of some of them, a cavity is produced



in the middle, and the lateral hyphae gradually grow up through the stroma towards the surface, whilst from the base and sides of the swollen lower portions numerous septate filiform hyphae—the paraphyses—arise; these grow up between the ascogenous hyphae, and fill the cavity of the perithecium. The stouter ascogenous hyphae are still seen at the base, and in larger numbers than before; these, apparently by branching, give rise to the asci, which eventually line the perithecium in great numbers (cf. Figs. 24–26). The complicated nature of the perithecium at this stage, its thick wall and dense mass of paraphyses, make this point very difficult to demonstrate clearly, but a comparison of the stages observed in *Poronia* with those figured by Fisch<sup>1</sup> in *Polystigma* and *Xylaria*, makes it probable that the course of events is as described above.

The mature perithecium consists of a very definite wall of closely interwoven hyphae lined with a smaller-celled hymenial layer, whence arise the very numerous club-shaped asci, intermingled with numerous paraphyses. The somewhat long neck, which opens by an ostiole to the exterior, is lined by delicate paraphyses, which more or less completely fill the cavity leading into the perithecium (Fig. 26).

It has been pointed out to me by Professor Marshall Ward that the observations at this stage suggest the possibility that the trichogyne-like hypha referred to above may be a sort of pioneer hypha to prepare the ostiole, and that it starts the dissolution of the surrounding hyphae and so prepares the passage.

A somewhat similar theory to the above was propounded in 1888 by Lindau<sup>2</sup>, in reference to the trichogynes developed by various Lichen-forming Fungi. At that time he regarded the trichogyne as partly sexual and partly mechanical in its function, but in 1899<sup>3</sup> he denied to the trichogyne, in the cases investigated by him, any sexual function, and described

<sup>1</sup> Fisch, Beiträge zur Entwicklungsgeschichte einiger Ascomyceten. Bot. Ztg., 1882.

<sup>2</sup> Lindau, Ueber die Anlage und Entw. einiger Flechtenapothecien. Flora, 1888.

<sup>3</sup> See Darbishire, Pringsh. Jahrb. xxxiv, Heft 2, p. 330.

it as a purely mechanical organ, which bores through the overlying tissues, preparatory to the subsequent growth of the paraphyses of the developing apothecium.

Darbishire<sup>1</sup>, in his recent work on *Physcia pulverulenta*, rejects Lindau's view for that Lichen, mainly upon the grounds of the unsuitable structural character of the trichogyne as a boring organ, and of the relative positions of the organic apex of the young apothecium and the line of growth of the trichogyne. These, he points out, are most frequently in quite different vertical planes, and therefore the trichogyne cannot prepare the way for the growth of the paraphyses, which he regards as the true boring or dissolving organs. Whilst on the one hand this objection of the relative position of trichogyne and perithecium will not hold in *Poronia*, on the other hand it seems impossible to relegate to the organ any function as an 'organ of conception,' for, in the first place, no trace of spermogonia with spermatia has been found in this Fungus, and in the second place, the only other form of spore which occurs besides the ascospores is that of the conidia which precede the ascospores, and which germinate readily, producing a cycle of development quite indistinguishable from that resulting from the germination of an ascospore. Hence, if the trichogyne-like process in *Poronia* is rightly regarded as the homologue of the similar organ occurring in the Florideae and the Collemaceae—and this seems the only tenable view—we may, so far as concerns its relation to a fertilization-process, regard it as a degenerate organ, reminding us of the functionless fertilization-tubes formed by the antheridia of some species of the Saprolegnieae. It would then be quite intelligible, if, having lost this sexual function, the organ should become more mechanical in its action. Undoubtedly (as Darbishire contends for *Physcia pulverulenta*) in *Poronia* the chief part of the work of boring through or dissolving the overlying hyphae is performed by the hyphae forming the lateral wall and the periphyses, but

<sup>1</sup> See Darbishire, Ueber die Apothecienentwicklung der Flechte *Physcia pulverulenta*. Pringsh. Jahrb. xxxiv, Heft 2.

it is conceivable that in their growth they follow a path already prepared for them, by the trichogyne-like hypha.

The *Asci* in their youngest stages are small club-shaped bodies with very abundant finely granular protoplasm, especially dense in the apical region, near which lies the large nucleus. The ascus gradually elongates; the nucleus divides into two, four, and then eight, and around these the protoplasm aggregates. As described by Harper<sup>1</sup> in various Ascomycetes, the ascospores arise by free cell-formation, some protoplasm being left over as an 'epiplasmic' layer, lining the wall of the ascus. This author regards such a mode of development as strong evidence against any close relationship between asci and sporangia (in which spore formation is by cleavage of the protoplasm, proceeding inwards from the sporangium walls), such as Brefeld's theory requires.

In spite of numerous trials, I have failed to get perfectly satisfactory karyokinetic figures in the division of the nucleus in the ascus, owing to difficulties in fixing and staining, but sufficiently convincing examples have been seen to show that two and then four nuclei are present, and that later each spore contains one nucleus. In the stage with the eight naked masses of protoplasm the free nuclei were not however clearly seen. The eight spores finally come to lie in a single row, each surrounded by a double wall, the outer of which, on ripening, becomes dark brown in colour, with a clearly marked lateral slit-like pit<sup>2</sup> (Figs. 28-30). When first liberated from the ascus, the elliptical spores are surrounded by a distinct gelatinous covering, resembling a halo. At the apex of the ascus the wall is seen to be folded in, causing the 'epiplasm' to form three peg-like projections, like those represented by Zopf in *Hypocopra*<sup>3</sup>. These are particularly obvious in the younger asci.

The *Conidia*, as already indicated, arise by abstriction from the ends and sides of the terminal hyphae of the stromata.

<sup>1</sup> Harper, Cell-division in Sporangia and Asci. Ann. of Bot., Dec., 1899.

<sup>2</sup> This lateral marking has been omitted by Brefeld in his figure of these ascospores, suggesting that his drawing may have been made from the other side of the ascus. Heft x, Taf. ix, Fig. 8.

<sup>3</sup> Zopf, Die Pilze, p. 92, Fig. 60.



The conidium-forming hyphae are divided into very small cells by numerous septa, and they tend to break down into these component cells, and as a result this layer of hyphae falls away during the formation of perithecia (Figs. 31, 32). This phenomenon was pointed out by Tulasne, Brefeld<sup>1</sup>, and Von Tavel<sup>2</sup>.

If sections of stromata of *Poronia* of various ages be treated with strong potash at 160° C. and stained with iodine and sulphuric acid, in the manner described by Wisselingh<sup>3</sup>, it is seen that the walls of the hyphae all contain chitin, with the possible exception of the outermost rind-like layer, which retains its original brown colouration.

After a study of the internal anatomy of *Poronia punctata*, one is perhaps most struck by its many points of resemblance with *Polystigma rubrum* and *P. fulvum*; this is of course especially noticeable in the form of the archicarp, which in *Polystigma* consists of 'a long coiled row of many cells,' one extremity of which projects above the surface as a trichogyne. The coil appears to divide up into portions, which, after distribution over the hypothecium, branch to form the ascogenous hyphae. The marked point of difference between the two genera is the formation in *Polystigma* of spermogonia with spermatia, whilst in *Poronia* degeneration or development, as the case may be, has resulted in the entire absence of these bodies. In *Polystigma*, however, the union of spermatia with the trichogyne has not yet been observed.

It should be pointed out in this connexion, that although, in *Poronia*, perithecia with ripe asci have been formed on stromata grown in captivity, yet the number of perithecia developed was smaller than in those stromata which had reached the stage of immature ascospores before the material was brought into the laboratory. Also, whilst the former took at least one month to form asci, in the latter specimens the spores ripened in a few days. We are, of course, unable to say how long is required for the earlier stages in the

<sup>1</sup> See Brefeld, l. c., Heft x, p. 261.

<sup>2</sup> Von Tavel, *Vergleichende Morphologie der Pilze*, p. 92. Jena, 1892.

<sup>3</sup> Wisselingh, *Pringsh. Jahrb.*, 1898.

formation of perithecia in nature. Up to the present, in stromata grown in pure cultures, only the very early stages have been observed, i.e. the small-celled knot of hyphae, enclosing the coiled Woronin's hypha, although several specimens are more than two months old, and have been supplied with free access of air, and grown under varied conditions of illumination and temperature. Possibly the time of year is the explanation of this check in the development, and the asci are by some special adaptation of the Fungus only capable of being formed in the autumn months. This point can naturally only be decided by a further continuation of the cultures throughout the coming year. Whatever may be the true explanation of the details in the formation of the perithecia of *Poronia*, it seems clear that in this genus we have a very interesting example of Fungi with a Woronin's hypha, which as De Bary has already pointed out<sup>1</sup> form a connecting link between the archicarps of *Collema* and *Polystigma* and the sporocarps of *Claviceps*. If we therefore regard these archicarps on the one hand as the homologues of the Woronin's hyphae on the other hand, we can give the position of *Poronia* as intermediate between *Polystigma* and *Xylaria*. In *Polystigma* the stroma is a somewhat less defined structure than in *Poronia*<sup>2</sup>, i.e. it consists of a fleshy cushion-like mass of tissue on the surface of the infected leaf, and in this mass the perithecia are embedded, whilst on the contrary the stromata of *Xylaria* are still more definite, more elaborate and horny structures than in *Poronia*.

This suggested position of *Poronia* is illustrated by a comparison of the details of the archicarp and its homologue and by the consideration of any other reproductive bodies which are formed in the respective genera ; thus :—

In *Collema* we find a coiled archicarp with a prominent trichogyne, which penetrates to the outer surface of the thallus, and there fuses with the spermatia, which are formed in

<sup>1</sup> De Bary, loc. cit., p. 236.

<sup>2</sup> Cooke, Handbook of British Fungi, p. 803.

definite organs, the spermogonia—that is, in this genus, there is a clearly marked sexual act. These facts have recently been confirmed by Bauer<sup>1</sup>.

In *Polystigma*, a similar coiled archicarp with a trichogyne is present. Here, too, are found spermogonia with spermatia, which were seen by Fisch<sup>2</sup> adhering to the trichogyne, but the union of the two was not observed.

In *Poronia*, we again find a coiled hypha (quite comparable to the archicarp of *Polystigma*), with a more or less degenerate trichogyne-process. In this genus, however, the spermogonia with spermatia are not developed, so that there can be no question of the sexual function of the trichogyne.

In *Xylaria*, this functionless trichogyne appears to have been dispensed with, as well as the spermogonia and spermatia. If this series of forms, and the implied homology of the organs in question be a correct view of the case, we must regard the ascospores of *Poronia* as formed parthenogenetically by intermediate stages from an organ, which represents the archicarp, which in certain other forms is sexually functional, but which in *Poronia* has lost that power. If further details of the stages between the coiled Woronin's hypha and the ripe asci could be determined, it is conceivable that later fusions of nuclei might be found to occur, which might be regarded as a secondary sexual act replacing the more obvious one, which has been lost in the earlier stage. This, presumably, would accord with Dangeard's<sup>3</sup> view, that the fusion of the two original nuclei in the young ascus represents the true sexual act in the various Ascomycetes, which he investigated<sup>4</sup>. No such fusions have been seen, however, in *Poronia*.

THE BOTANICAL LABORATORY, CAMBRIDGE,  
March, 1900.

<sup>1</sup> Bauer, Zur Frage nach der Sexualität der Collemaceen, Ber. d. Deutsch. Bot. Ges., vol. xvi, 1898.

<sup>2</sup> Fisch, loc. cit.

<sup>3</sup> For a full discussion of the views of Harper and Dangeard upon the Sexuality of the Ascomycetes, see Wager, On the Sexuality of the Fungi, Ann. of Bot. xiii, Dec., 1899.

<sup>4</sup> See Dittrich, Cohn's Beiträge, viii, 1898.



# EXPLANATION OF FIGURES IN PLATES XIV AND XV.

Illustrating Miss Dawson's paper on *Poronia punctata*.

## PLATE XIV.

Fig. 1. Stromata of *Poronia punctata* from nature. *a*, stromata-stalks embedded in the substratum; *b*, stromata raised above the substratum.

Fig. 2. Group of individual stromata of different types, drawn from nature.

Fig. 3. Series of stages of growth of a group of stromata, cultivated in a pure state from an ascospore, on horse-dung extract in gelatine and agar. Ascospore sown on Nov. 4. *a*=Nov. 23; *b*=Nov. 27; *c*=Nov. 29; *d*=Dec. 1; *e*=Dec 5; *f*=Dec. 8. T.=15° C. (Nat. size.)

Fig. 4. Stages in growth of stromata with disk-shaped heads, from germinating ascospore, sown Nov. 18. *a*=Nov. 28; *b*=Dec. 3; *c* and *d*=Dec. 11, showing two portions of culture, drawn separately; *e*=Jan. 17. This culture transferred from gelatine medium to cotton-wool on Nov. 28. (Nat. size.)

Fig. 5. Branched stroma, grown in pure culture, on cotton-wool medium, from conidium germinated in gelatine medium. This stroma formed in eight days, after the culture was aerated and kept in the light. T.=17°. (Nat. size.)

Fig. 6. Proliferation of head of stroma, after apex has been cut off. *a*=Feb. 23; *b*=Feb. 26; *c*=Mar. 1; *d*=Mar. 5. T.=10°-15° C. (This represents later stages in the development of the stroma to the extreme left of Fig. 4 *e*.) (Nat. size.)

Fig. 7. Two unusually large stromata grown up on culture drawn in Fig. 4 *e*, after increased aëration and lower temperature. Age, Jan. 17-Feb. 26. T.=10°-12° C. (Nat. size.)

Fig. 8. Low power drawing of individual young stroma, as shown in Fig. 3 *a*, nineteen days after sowing of ascospore. Horizontal mycelium flattened parallel to erect stroma, crystals of calcium oxalate in substratum. ( $\times 12$ .)

Fig. 9. Crystals (Fig. 8) under obj.  $\frac{1}{4}$ .

Fig. 10. Portion of mycelium grown from conidium in hanging drop culture. Separation of crystals from substratum. Eleven days old. ( $\times 180$ .)

Fig. 11. Different methods of germination of conidia in gelatine, with horse-dung extract. ( $\times 350$ .)

Fig. 12. Consecutive stages in germination of conidium in hanging drop. *a*, Nov. 7, 1 p.m.; *b*, Nov. 8, 10.30 a.m.; *c*, Nov. 9, 10 a.m.; *d*, Nov. 10, 10 a.m.; *e*, Nov. 10, 3 p.m.; *f*, Nov. 11, 10 a.m.; *g*, Nov. 12, 8.45 a.m.

Fig. 13. Consecutive stages in germination of ascospore in hanging drop. Medium=gelatine with horse-dung extract. *a*=Nov. 10-12; *b*=Nov. 13, 11 a.m., t.=17°; *c*, 2.30 p.m., t.=18°; *d*, 5.15 p.m., t.=17°; *e*, 7.10 p.m., t.=17°; *f*, 10.15 p.m., t.=18°; *g*, 11.30 p.m., t.=18°; *h*, Nov. 14, 12.30 a.m., t.=18°; *i*, 5. ( $\times 350$ .)

Fig. 14. Mycelium from spore, shown in Fig. 13; Nov. 14, 10.30 a.m., t.=17°. ( $\times 100$ .)

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Fig. 15, *a* and *b*. Portions of mycelium grown from ascospore (Fig. 13) showing cross-connexions of hyphae. Nov. 14, 11 a.m. ( $\times 180$ .)

PLATE XV.

Fig. 16. Portion of head of stroma, showing perithecia in various positions, and of different ages. ( $\times 90$ .)

Fig. 17. Young stroma taken from extreme left of culture, shown in Fig. 4, *e*. Hardened in Hermann's solution. Stained with Rubin S. Deeply stained, swollen hyphae below apical regions. ( $\times 20$ .)

Fig. 18. Deeply stained swollen hyphae in stroma of age represented in Fig. 16. These hyphae especially abundant in outer regions of stroma. *a* ( $\times 150$ ); *b* ( $\times 1260$ .)

Fig. 19. First stages in coiled Woronin's hypha, before growth of investing hyphae. *a* ( $\times 230$ ); *b* ( $\times 340$ .)

Fig. 20. Perithecium showing Woronin's hypha, and trichogyne-process passing to exterior. Iron Alum, Haematoxylin. ( $\times 230$ .)

Fig. 21. Two consecutive sections of same perithecium showing coiled hypha and trichogyne. Coil becoming broken up, and form of perithecium less spherical. ( $\times 230$ .)

Figs. 22-25. Stages in development of perithecium, showing formation of central cavity, periphyses, paraphyses and ascogenous hyphae. ( $\times 170$ .)

Fig. 26. Mature perithecium, ostiole completely formed. Asci of different ages lining the hymenial layer. ( $\times 170$ .)

Fig. 27. Broad deeply stained hypha, grown up from amongst vegetative hyphae and coiled to form Woronin's hyphae. Surrounding investing hyphae smaller celled. Iron Alum, Haem. ( $\times 680$ .)

Fig. 28. Group of asci, from perithecium hardened in Abs. Alc. Asci in dilute ammonium hydrate. ( $\times 300$ .)

Fig. 29. Young asci separated out showing accompanying paraphyses. ( $\times 300$ .)

Fig. 30. Different stages in development of ascospores. *a* and *b* ( $\times 600$ ); *c* and *d* ( $\times 400$ .)

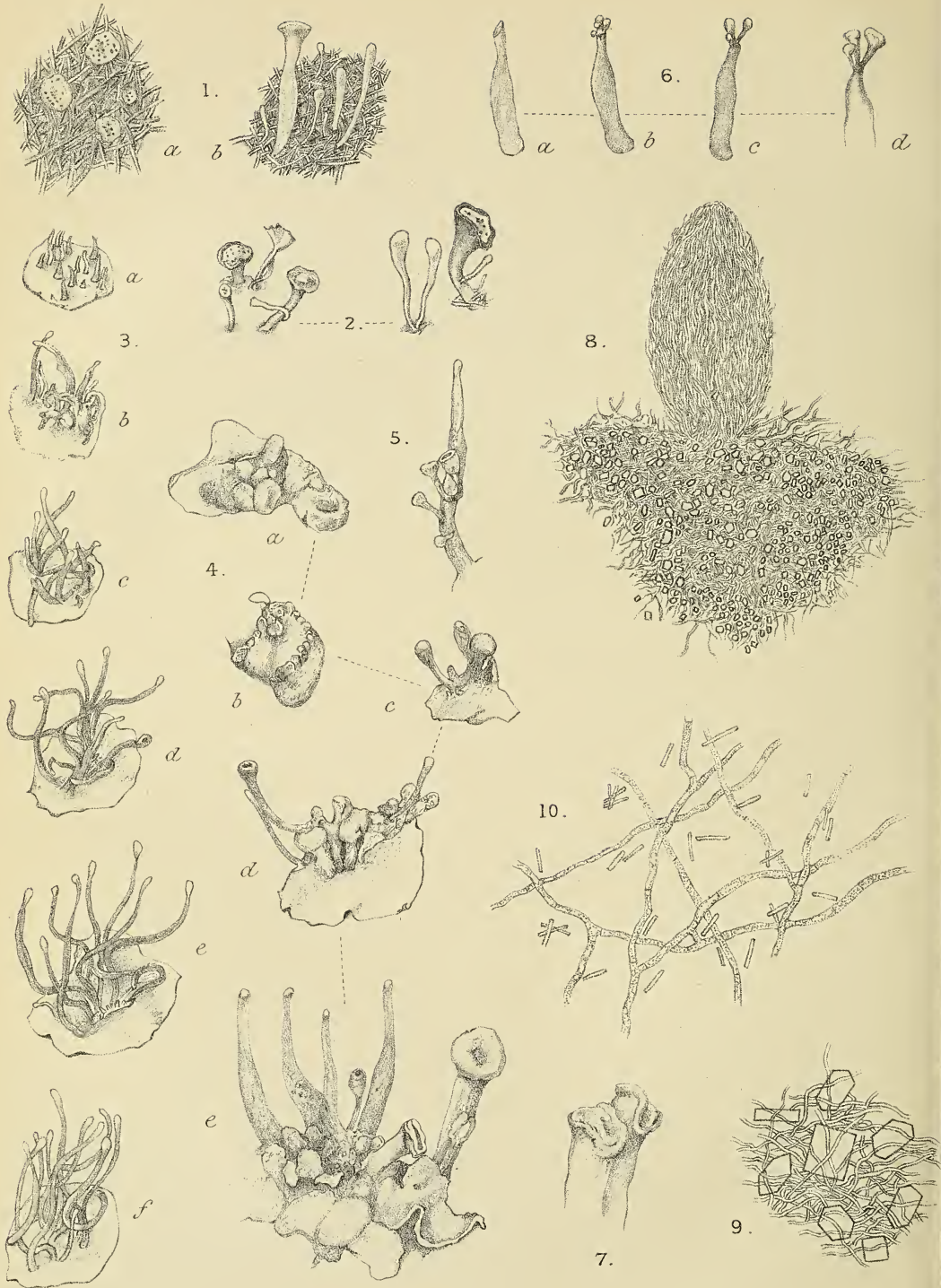
Fig. 31. Stroma grown in pure culture. Conidial formation from terminal hyphae. No perithecia forming. ( $\times 40$ .)

Fig. 32. Formation of conidia, breaking up of conidia-bearing hyphae. ( $\times 330$ .)

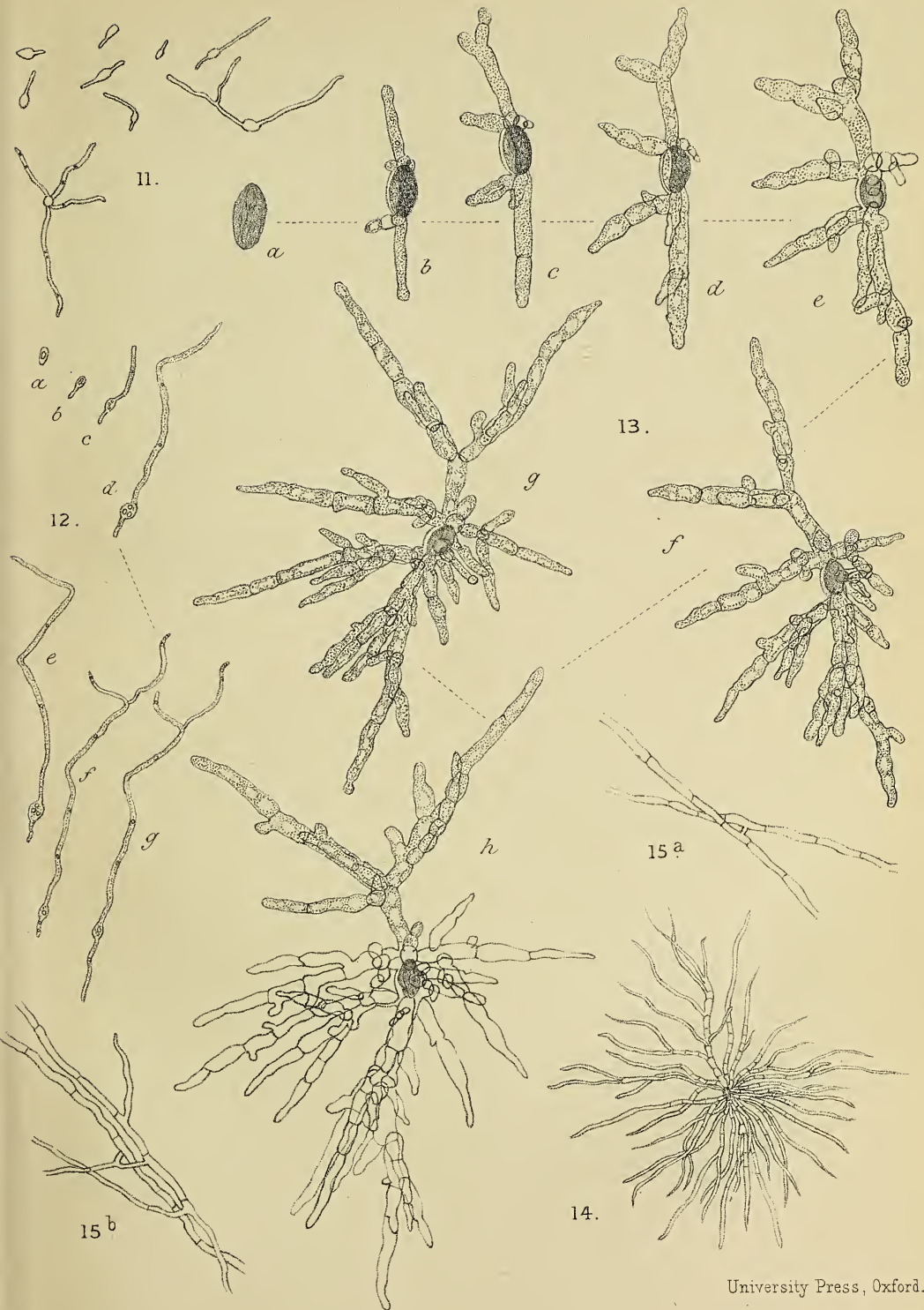
Fig. 33. Group of fresh ascospores, showing lateral slit-like depression of exosporium. ( $\times 320$ .)







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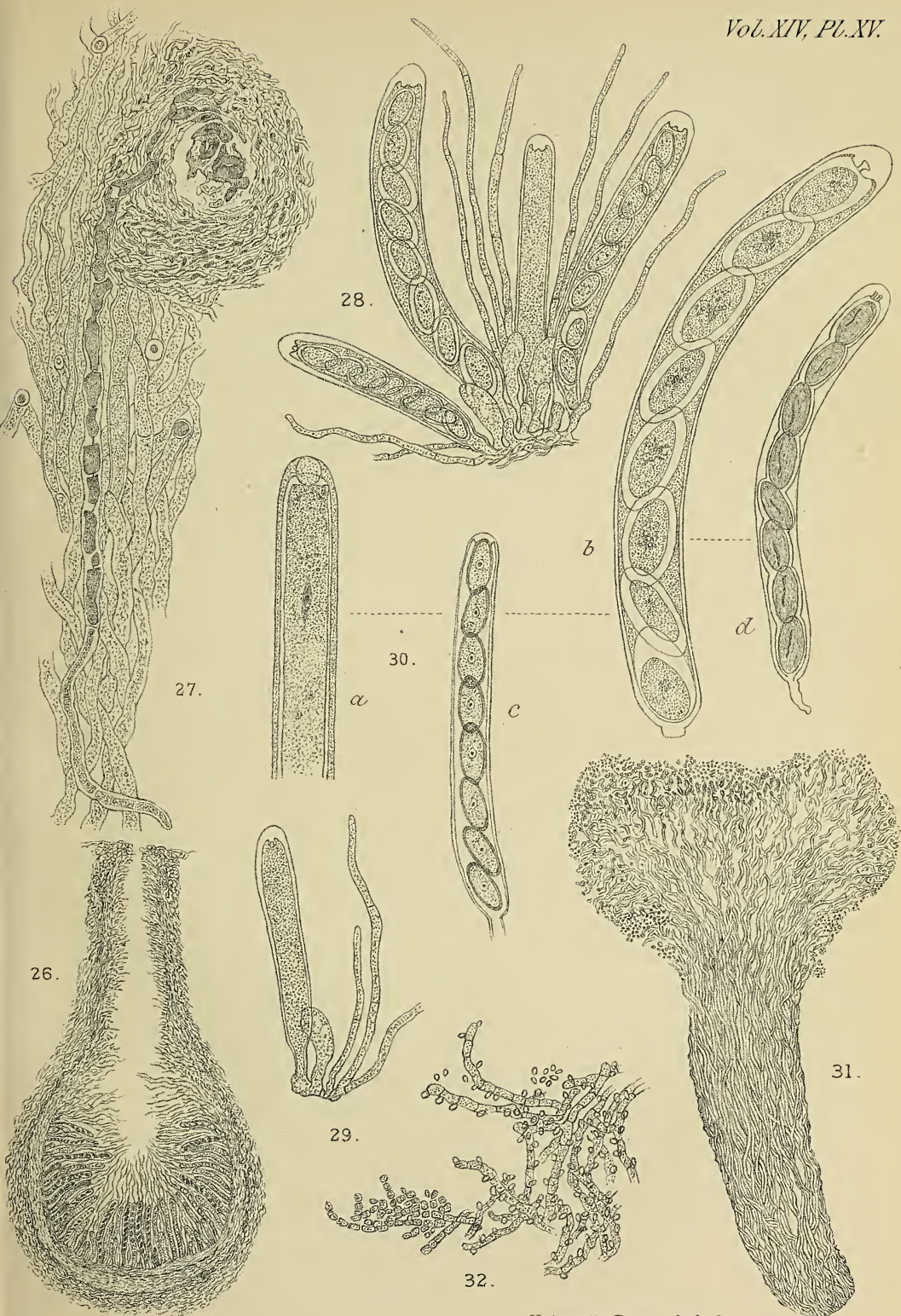






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# On the Fertilization of *Peronospora* *parasitica*.

BY

HAROLD WAGER.



With Plate XVI.



SINCE the classical researches of De Bary upon the phenomena of fertilization in the *Peronosporae*<sup>1</sup>, the subject has been studied by Marshall Ward<sup>2</sup>, Fisch<sup>3</sup>, Chmielewsky<sup>4</sup>, Istvanffi<sup>5</sup>, Dangeard<sup>6</sup>, Berlese<sup>7</sup>, Stevens<sup>8</sup>, and myself.

The investigations of De Bary and Marshall Ward definitely established the fact that certain genera of the *Peronosporae* exhibited some of the phenomena of fertilization in the fusion of protoplasmic masses derived from male and female cells.

<sup>1</sup> See Comp. Morph. &c., of Fungi, Eng. Ed.

<sup>2</sup> Observations on the genus *Pythium*. Q. J. M. S., xxiii, 1893.

<sup>3</sup> Ueber das Verhalten der Zellkerne in fusionierenden Pilzzellen. See Bot. Cent. xxiv, p. 221.

<sup>4</sup> Zur Frage über die Copulation der Kerne beim Geschlechtsprocess der Pilze. See Bot. Cent. xxxviii, p. 689.

<sup>5</sup> Ueber die Rolle der Zellkerne bei der Entwicklung der Pilze. Ber. d. deut. Bot. Gesell. xiii, 1895, p. 456.

<sup>6</sup> Recherches sur la Reproduction sexuelle des Champignons. Le Botaniste, 1894, p. 221.

<sup>7</sup> Ueber die Befruchtung und Entwicklung der Oosphäre bei den *Peronosporeen*. Jahr. f. wiss. Bot. xxxi, p. 159.

<sup>8</sup> The compound Oosphere of *Albugo Bliti*. Bot. Gaz. xxviii, 1899.

In *Pythium*, for example, it was shown that nearly the whole of the protoplasm in the antheridium passed over into the oosphere through the fertilizing tube. In *Phytophthora* a very minute quantity only was seen to pass over into the oosphere, and in the other genera the passage of protoplasm from the male to the female organ could not be seen.

At that time nothing was known of the part taken by the nuclei in this process. The presence of nuclei in the cells of the Peronosporae had been announced by Schmitz in 1879<sup>1</sup>, but their behaviour in the process of fertilization was first of all studied by Fisch in 1885<sup>2</sup>. He investigated the genera *Pythium* and *Cystopus*, and came to the conclusion that, in *Pythium*, the nuclei of the oogonium fuse together to form a single female pro-nucleus, that the antheridium contains a single nucleus probably also due to the fusion of several nuclei; and that in the process of fertilization the nucleus of the antheridium passes over into the oosphere and becomes fused with the female nucleus to form the single nucleus of the oospore.

These observations on *Pythium* have never been confirmed or refuted; and in the light of recent investigations it is very desirable that this genus should be thoroughly studied. This is the more important, as the imperfect observations made by Fisch upon fertilization in *Cystopus*<sup>3</sup>, which, he stated, presented, so far as could be seen, phenomena of fertilization similar to those observed in *Pythium*, have since been shown by more than one observer to be incorrect. His observations on the multinucleate character of the oogonium and antheridium in *Cystopus* have however been confirmed by all observers except Chmielewsky<sup>4</sup>, who described the oogonium and antheridium of *C. candidus* as uninucleate structures, and states that Fisch must have mistaken the knots in the protoplasmic network for nuclei.

<sup>1</sup> Untersuchungen über die Zellkerne der Thallophyten. Verhandlungen des naturhistorischen Vereins der preussischen Rheinlande und Westfalens, Bd. xxxvi, p. 345.

<sup>2</sup> loc. cit.

<sup>3</sup> loc. cit.

<sup>4</sup> loc. cit.



In 1889<sup>1</sup> I showed that in *Peronospora parasitica* both oogonium and antheridium are multinucleate; that previous to the differentiation of the oosphere the nuclei of both these organs undergo mitosis; that in the separation of the oosphere from the periplasm the majority of the nuclei of the oogonium pass into the periplasmic layer, and that finally the oosphere when ready for fertilization contains only one nucleus.

The exact details of the fertilization were not investigated, but the following conclusion was arrived at from a consideration of the facts observed: 'The passage of a nucleus from the antheridium into the oosphere has not been directly observed, but it is probable that fertilization does take place, as two nuclei have been seen in the oosphere at about the time when a nucleus or nuclei from the antheridium appear to pass over into the fertilizing tube.'

Istvanffi<sup>2</sup>, in 1889, described the oogonia and antheridia of *Cystopus Portulacae* as multinucleate, and referred to the process of fertilization as consisting probably in the fusion of the nuclei of the two sexual organs. 'Die Befruchtung erfolgt auch hier höchst wahrscheinlich nur durch die Vermischung der Zellkerne beiderlei Sexualorgane.'

In 1894 Dangeard<sup>3</sup> made some observations on *Pythium monospermum* Prings. and *P. proliferum* De Bary, in which he showed that the antheridia and oogonia are multinucleate. He also pointed out that a central oleaginous globule appears in the oogonium at a certain stage, and that this was probably mistaken by Fisch for a nucleus and led him to the conclusion that all the nuclei of the oogonium fused together to form a single sexual nucleus.

Dangeard also found that the antheridia and oogonia are multinucleate in *C. candidus*, *C. cubicus*, *Plasmopara nivea*, and *P. densa*. He was unable to observe any details of

<sup>1</sup> Wager, On the Nuclei of *Peronospora parasitica*, &c., Ann. of Bot., vol. iv, p. 127.

<sup>2</sup> A penészek sejtmagávról (De fungorum nucleis). Magyar Növénytani Lapok, xiii, 1889, pp. 33-46. See Ueber die Rolle der Zellkerne bei der Entwicklung der Pilze, Ber. d. deut. Bot. Gesell., Nov. 1895, p. 457.

<sup>3</sup> loc. cit.

fertilization, but he comes to the conclusion that in *C. candidus* some of the nuclei remain in the periplasm and contribute to the formation of the exospore, the others remain in the oosphere, and are there probably joined by the nuclei from the antheridium<sup>1</sup>. In *Plasmopara densa* he made the interesting observation that when the protoplasm concentrates towards the centre of the oogonium for the purpose of forming the oosphere, two small nuclei are visible at the centre quite close together; the other nuclei are found in the periplasm. He was, however, unable to say whether these two nuclei fuse together or not.

The question of fertilization in the Peronosporae remained at this stage until the appearance of my paper on *Cystopus candidus* in 1896<sup>2</sup>, in which I described in detail the differentiation of the oosphere, and the fertilization and maturation of the zygote. Previous to fertilization, all the nuclei, both of the antheridium and oogonium, undergo mitosis. One of these nuclei becomes separated from the others in the oogonium to form the nucleus of the oosphere, and this then fuses with a single nucleus from the antheridium, which passes into the oosphere through the fertilizing tube, to form the egg or zygote nucleus. This nucleus immediately divides, and by subsequent divisions thirty-two nuclei are formed, so that the ripe zygote is multinucleate.

These results were confirmed by Berlese<sup>3</sup> on *Cystopus Portulacae*, and he further extended them to various species of *Peronospora*, *P. Ficariae*, *P. Alsinearum*, and *P. effusa*. His observations confirm my own in every important and essential detail, except as to the condition of the nuclei in the actual fusion. In *C. candidus* I pointed out that the fusion takes place in the resting stage of the nuclei. In *C. Portulacae* and *Peronospora Ficariae*, Berlese's figures indicate the same thing; but in his figures of *P. Alsinearum*

<sup>1</sup> loc. cit., p. 128.

<sup>2</sup> On the Structure and Reproduction of *Cystopus candidus* Lev., Ann. of Bot. x, 1896.

<sup>3</sup> loc. cit.

and *P. effusa* he shows a state of fusion in which the fusing nuclei are similar to those in *Ascaris*, in which the fusion takes place in the chromosome stage, and the chromosomes are visible before fusion; and he refers generally to the process of fertilization in the following words as if it referred to the whole group: 'Auch die Chromosomen werden gut sichtbar, und ich konnte auch im Spermakern 12-16 derselben nachweisen; die Kernmembran verschwindet allmählich und es beginnt die Verschmelzung der beiden Kerne. . . . Es geht mit Sicherheit aus meinen Untersuchungen hervor, dass der embryonale Kern aus einer Zahl von Chromosomen zusammengesetzt ist, die um die Hälfte grösser ist als die Zahl der Chromosomen, welche in einem jeden der beiden Geschlechtskerne, die ihn zusammensetzen, nachweisbar ist, und dass dieselben vor der Befruchtung gar keine Reduction zeigen.'

With this exception, however, Berlese and I are in agreement as to the essential facts of fertilization in *Cystopus*, and it now remained for me to determine whether my former observations on *P. parasitica*<sup>1</sup>—that the formation of the female or oosphere-nucleus takes place by the fusion of two or more periplasmic nuclei, and that the ripe oospore is uninucleate—were correct, or whether the facts are in accord with the more recent observations of Berlese on *Peronospora*, and especially with his statement that the ripe oospore of *P. parasitica* is multinucleate<sup>2</sup>. I found on reinvestigation that the process of fertilization is similar in its essential features to that which takes place in *C. candidus*, but that, unlike all the other species of *Peronospora* and *Cystopus* which have so far been examined, the ripe zygote is *uninucleate*.

Stevens<sup>3</sup> has recently published an account of the fertilization in *Cystopus (Albugo) Bliti*, which differs entirely in its essential details from that described by Berlese and myself for other members of the group. The oogonium and antheridium

<sup>1</sup> Annals of Botany, vol. iv, Nov., 1889.

<sup>2</sup> Berlese, loc. cit. p. 181.

<sup>3</sup> loc. cit.



are multinucleate and agree in their general structure with those of the species already described. The protoplasm becomes differentiated into an oosphere and periplasm. All the nuclei pass into the periplasm and there undergo mitosis. The oogonium contains about 250 nuclei, the antheridium about 35, which is a larger number than occurs either in *C. Portulacae* or *C. candidus*. So far the observations are, with differences in details, generally in accord with those made by previous observers; but he now goes on to point out, after describing in detail the process of nuclear division in the oogonium, that 'those dividing nuclei that lie tangential to or wholly outside of the boundary line between the ooplasm and periplasm leave their daughter-nuclei in the periplasm. Each of the spindles which cross the line (boundary between oosphere and periplasm) gives one daughter-nucleus to the oosphere and the other to the periplasm, and the line of differentiation is sharply defined and unmistakable.' 'As a result of the division a large number of nuclei pass into the ooplasm, thus producing a multinucleate cell (oosphere) containing by actual count an average of forty-five to fifty-five nuclei.' Thus 'the mature oosphere contains many female nuclei, and fertilization is effected by the discharge of many male nuclei from the antheridial tube and their subsequent fusion with the female nuclei in pairs. An oospore results from this multiple sexual act with about one hundred fusion nuclei, which remain in the resting condition until germination.'

These observations are exceedingly interesting, as they indicate that the process of fertilization in the Peronosporaeae is not necessarily the same for all genera or for all species even of the same genus. It becomes very important, therefore, that the process of fertilization in this group should be studied in as many forms as possible. Up to the present time four different methods of fertilization in the Peronosporaeae have been described. One of these, described by Chmielewsky for *Cystopus candidus*, has been shown to be incorrect. The others still stand. These three methods are—

1. Collective nuclear fusion as in *Pythium* (Fisch).
2. Binuclear fusion as in *Cystopus candidus*, *C. Portulacae* and species of *Peronospora* (Wager and Berlese).
3. Multiple nuclear fusion, in pairs, as in *C. Bliti* (Stevens).

It is probable that the method of fertilization described by Fisch for *Pythium* will be found to be untenable. The second type of nuclear fusion has been found to be the most frequent in the Peronosporae, so far; and the third type must be regarded therefore, at present, as the anomalous one.

The observations on *P. parasitica* which follow show that this species comes definitely in the second group. As the general structure of the Fungus was described in a previous paper I shall confine myself here to a description of the sexual organs and the process of fertilization.

As in *Cystopus*, the nuclei of the young oogonium of *Peronospora parasitica* are irregular in shape and the outline of the oogonium, before it is cut off from the parent hypha, presents a somewhat crumpled appearance; the nuclei are often elongated in the direction of the flow of the protoplasm into the oogonium as in *Cystopus candidus*, and as Stevens has also shown to be the case in *C. Bliti*. As soon as the partition-wall separating the oogonium from the hypha is formed, the oogonium expands and becomes more or less spherical; the nuclei at the same time regain their spherical shape (Plate XVI, Fig. 1).

The structure of the nuclei at this stage is simple. They possess a nuclear membrane and a granular network, but no nucleolus, or if a nucleolus is present it is very small and difficult to distinguish from the granules of the network. The structure of the nuclei of the antheridium is the same.

Soon after the delimitation of the oogonium the protoplasm exhibits a vacuolar structure; the antheridium becomes closely attached to the wall of the oogonium and, as I have shown is also the case in *Cystopus*, a granular mass of protoplasm appears just beneath the membrane of the oogonium at the place where the fertilizing tube will be formed. At this point the wall separating the oogonium from

the antheridium becomes very thin and a slight hyaline protoplasmic papilla becomes formed which tends to push itself into the antheridium (Fig. 1). The same structure occurs in *C. candidus*, but is more prominent, and in *C. Bliti* Stevens has shown that it is still more highly developed; for the protoplasm pushes itself actually into the antheridium and there forms a very conspicuous swollen papilla inside it<sup>1</sup>.

Whatever may be the exact explanation of this curious structure, it appears to be connected in some way with the perforation of the oogonial wall and the formation of the fertilizing tube. It is much less highly developed in *C. candidus* and *P. parasitica* than in *C. Bliti*. I have called it the receptive papilla, because it marks the place where the penetration of the fertilizing tube takes place. Stevens is inclined to give it a more important significance than this; but it appears to me to be distinctly homologous with the receptive spot of other ovum-cells and to perform a similar function. Such receptive spots or papillae are found in the oogonia of *Vaucheria* and *Oedogonium*, and are well marked differentiations in the protoplasm.

As soon as the oogonium is formed the nuclei begin to increase in size; the linin-network and chromatin-granules become more prominent and stain more deeply. The cytoplasm loses at the same time, to some extent, its power of taking up stains, and the vacuoles become larger and less numerous. Then a differentiation of the protoplasm into ooplasm and periplasm begins, and all the nuclei pass into the periplasm (Fig. 2). They here undergo mitosis. The two regions are distinctly marked off from one another, although as yet there is no partition-wall between them. The periplasm is a granular, homogeneous layer at the periphery of the oogonium. The ooplasm is a vacuolate spherical mass of cytoplasm in the centre (Figs. 2, 3, 4). The nuclei at this stage are often arranged in a single regular layer immediately around the periphery of the ooplasm<sup>2</sup>.

<sup>1</sup> loc. cit. p. 154 (see Plate XIII, Figs. 47-54).

<sup>2</sup> See Fig. 8, Plate VI, Ann. of Bot., vol. iv, 1889.



The changes just described differ from those observed in *Cystopus*. In *C. candidus* the protoplasm first becomes separated into a peripheral vacuolar portion and a central denser protoplasm containing all the nuclei and smaller vacuoles. Then the central mass separates into a vacuolar ooplasm and a non-vacuolate granular layer of periplasm, into which all the nuclei pass. Stevens describes a somewhat similar phenomenon in *C. Bliti*<sup>1</sup>, where the protoplasm of the young oogonium is irregularly vacuolate. In the process of differentiation of the oosphere accumulations of denser protoplasm are found, separated from one another and the wall of the oogonium by vacuoles of varying sizes. These denser protoplasmic masses coalesce and the vacuoles become restricted to the periphery, so that we get, as in *C. candidus*, a central denser mass of protoplasm surrounded by a vacuolar layer. The nuclei pass to the periphery of the central mass, and then the periplasm becomes distinctly differentiated as a dense granular layer around and between the nuclei. The condition in which there is a distinct line of demarcation between periplasm and ooplasm, but no definite wall between the two, is called by Stevens 'the stage of zonation' (p. 157).

As the nuclei pass into the periplasm they undergo changes which lead to division. They increase in size and the chromatin-granules fuse together to form the chromosomes, deeply staining granules, which accumulate in the equatorial plane. A spindle is formed, the nuclear membrane disappears, and the chromosomes become divided into two groups which separate to become the daughter-nuclei. No nucleolus is visible during the mitosis and no definite centrosomes, although occasionally granules at the poles of the spindle were observed which might have been taken for such. The nuclear division described by Stevens in *C. Bliti* agrees in its essential details with this, except that the nucleolus persists until a late stage in the division and the nuclear membrane does not disappear so soon. The nuclei of the antheridium appear to divide simultaneously with those in the oogonium.

<sup>1</sup> loc. cit.

During the nuclear division the cytoplasm of the oosphere undergoes changes. The larger vacuoles disappear and smaller ones take their place; the central cytoplasmic mass becomes vacuolate; and, finally, the whole of the protoplasm of the oosphere exhibits a distinct foam-structure due to the large number of small vacuoles which now take the place of the larger ones, of which none, or only one or two, may remain (Figs. 5, 6, 7).

As this vacuolization is taking place a central homogeneous body makes its appearance (Figs. 2-7). This appears to be formed by the gradual condensation of a mass of granular cytoplasm in the centre or near the centre of the oosphere (Figs. 2, 3); and when it is completely formed it stains more deeply than the surrounding cytoplasm (Figs. 4, 5, 6). This structure has been known for some time, but it was first correctly described and its probable function indicated in my paper on *C. candidus*<sup>1</sup>. It was, as I have previously pointed out, probably this structure which was described by Dangeard<sup>2</sup> as an oil drop and by other observers as a nucleus. Swingle confirms my description of it, and suggests that it is a new organ or organoid of the cell<sup>3</sup>. In my original description of it I pointed out that shortly after its appearance one of the nuclei produced by division in the oogonium comes into close contact with it and gradually becomes more or less embedded in it; the fertilizing tube also grows directly towards and comes into contact with it. This indicates that it may in some way or other exert an attraction first upon the female nucleus, and secondly upon the fertilizing tube, thus helping to bring the sexual nuclei together. Stevens, who finds a similar structure in *C. Bliti*, *C. Tragopogonis*, and *C. Portulacae*, believes that we have here an organ of the oosphere which may be of the nature of a dynamic centre. He proposes to call it a *coenocentrum*. As I pointed out, however, in *C. candidus*, and as Stevens shows in *C. Bliti*, it is not a permanent organ of the cell. It disappears in *C. can-*

<sup>1</sup> loc. cit.

<sup>2</sup> loc. cit.

<sup>3</sup> Two new Organs of the Plant Cell. See Bot. Gazette, 1898, p. 110.

*didus* soon after fertilization, as Swingle has also shown<sup>1</sup>, and in *C. Bliti* it reaches its maximum development when the daughter-nuclei of the first mitosis pass into the oosphere, but disappears before fertilization takes place<sup>2</sup>. In *P. parasitica*, as in *C. candidus*, it attains its highest development at the time when fertilization is just about to take place (Fig. 6); after fertilization it begins to degenerate, and finally disappears (Figs. 8, 9). That it is not concerned actually in the fusion of the sexual nuclei, but only in bringing them together, is indicated by the fact that in *P. parasitica*, although it is present when the nuclei come together for the first time, it has completely disappeared before fusion takes place (Figs. 9-14). In *C. candidus*, where the nuclear fusion takes place immediately after the male nucleus enters the oosphere, the coenocentrum does not entirely disappear until the fusion is complete. All the evidence therefore before us, as to the function of this structure, indicates that it is functional in bringing the sexual nuclei together; and from the fact that in *P. parasitica* the female pro-nucleus becomes elongated in the direction of the coenocentrum, as it passes towards it through the oosphere (Fig. 4), and that the fertilizing tube may grow directly towards it (Fig. 7), it appears that it may exert an attractive force of some kind by which the sexual nuclei are brought into contact, and that as soon as this has been accomplished it begins to disappear.

As soon as this central body has made its appearance, one of the nuclei from the periplasmic layer comes into close contact with it (Figs. 4-7). This is the female pro-nucleus. In my former paper I stated that this nucleus was formed by the fusion of two nuclei, but this is not supported by my recent observations, and I suspect that I was misled by the coenocentrum, which at this stage looks very much like a nucleus, especially in badly stained specimens. This nucleus becomes elongated in the direction of the coenocentrum as it passes through the cytoplasm, and when it reaches the coenocentrum it is often spindle-shaped or fusiform (Fig. 4). Even

<sup>1</sup> Swingle, loc. cit.

<sup>2</sup> Stevens, loc. cit. p. 162.



in the periplasm it is often elongated; and I have sometimes seen more than one nucleus in the periplasm elongated in the direction of the coenocentrum: but so far as I have been able to observe, only one nucleus actually comes into contact with it. The ooplasm at this stage is finely vacuolate and possesses in consequence a very distinct foam-structure (Fig. 5).

The fertilizing tube penetrates the oogonium at the place where the receptive papilla is formed. It forms at first a thin-walled spherical or oval sac in contact with the periplasm (Figs. 4, 5). It then penetrates the oosphere by means of a thin-walled tubular portion which is pushed out at its apex (Fig. 7). The fertilizing tube then presents a characteristic appearance, consisting of a thin-walled tubular portion in the oosphere and a slightly expanded, more or less spherical portion, in the periplasm. There are no nuclei in the tube when it is first formed, but before it has pushed itself into the oosphere, one nucleus from the antheridium passes into it, and as it penetrates the oosphere a second nucleus may pass into it (Fig. 7).

Just before it passes into the fertilizing tube, the antheridial nucleus changes its shape, becoming slightly elongated and often pointed at the anterior end (Fig. 4); this apparently enables it to pass more easily through the narrow opening which leads from the antheridium into the fertilizing tube.

When the apex of the fertilizing tube comes into the neighbourhood of the central mass, with which the female nucleus is in contact, it opens and the male nucleus passes through the aperture into the oosphere and comes into contact with the coenocentrum (Figs. 7, 8). The male nucleus is slightly smaller than the female nucleus and stains differently in the carmine-nigrosin stain; the male nucleus is stained red, the female nucleus blue. They come into close contact, but do not fuse at once; they gradually increase in size, the male more rapidly than the female, until they are approximately equal, and then separate to opposite sides of the

cell (Fig. 9). In this position they become more deeply stainable, and consequently more conspicuous. Their structure exhibits a granular network, with deeply stained granules, and in the later stages a nucleolus can be made out.

Meanwhile the wall of the oospore, which was beginning to form just at the time of fertilization, has been increasing in thickness and forms now a very definite membrane, surrounded by the periplasm and the rest of the oogonial nuclei. The protoplasmic contents lose their fine vacuolate structure. The vacuoles decrease in number and increase in size. They mark the position of the large oil-drops which are seen in the living oospore. The cytoplasm at this stage stains less intensely than before, but the two nuclei, which are now very conspicuous objects in the cell, stain more deeply, having apparently taken up the stainable substances of the cytoplasm.

The fusion of the two nuclei now takes place (Figs. 10-15). They gradually approach again and come into close contact. The wall between them breaks down and the contents of the two nuclei fuse together. First they are dumb-bell shaped, then oval, and finally, when the fusion is complete, spherical (Fig. 16). The nucleoli remain distinct until the fusion is nearly completed, they then fuse together to form a single conspicuous nucleolus which stains deeply. The fusion definitely takes place in the resting stage of the nuclei; there is no indication whatever of chromosomes, as figured by Berlese in *P. Alsinearum* and *P. effusa*.

The retarded nuclear fusion just described is not uncommon. It may occur in the zygotes of *Spirogyra*, *Cosmarium*, *Closterium*, *Basidiobolus*, and *Polyphagus*, and, in some cases, may even not take place until germination has commenced.

In the *Peronosporae* we have the two types of fusion. In *C. candidus*, as I have shown, the nuclei fuse at once, immediately after the entry of the male nucleus into the oosphere, and the same thing also occurs in *C. Portulacae*, *P. Ficariae*, *P. Alsinearum* and *P. effusa* according to Berlese. *P. parasitica* is at present the only member of the group with retarded

nuclear fusion; and in this species it is delayed until the thick zygote-membrane has been partly formed.

At the time the fusion takes place the protoplasm of the oospore contains numerous oil-drops. These gradually fuse together to form a large central oil-globule of weak refringent power. The protoplasm fills the space between the oil-drop and the membrane. It is a finely granular substance and contains, as De Bary<sup>1</sup> pointed out, 'a round or elongated and perfectly pellucid spot' close to the membrane on one side of the spore. De Bary surmised that this might be the nucleus, and this turns out to be correct. The ripe oospore of *P. parasitica* is uninucleate; the nucleus is found in the position indicated by De Bary and is clearly visible in the living condition. In all the other species of *Peronospora* which have been examined the ripe zygote is multinucleate, and in *Cystopus*, so far as we know at present, the ripe oospores of all species are multinucleate. Whether the zygote of *P. parasitica* becomes multinucleate just before germination takes place I do not know, as I have not yet been able to get them to germinate.

The periplasm and periplasmic nuclei remain visible for some time but ultimately become transformed into the exosporium, which is laid down on the surface of the endosporium as a coarse network (Fig. 17). The wall of the oogonium persists, and as the oospore ripens it becomes much thickened and serves as an additional protective layer.

The phenomena of fertilization in *P. parasitica* thus described conform, in their essential characteristics, with those observed in the higher plants and animals, so far as the actual fusion of nuclei is concerned; and it resembles in a striking manner the fusion of nuclei in *Coleochaete pulvinata* described by Oltmanns<sup>2</sup>. Unfortunately however it has not been possible to arrive at any definite conclusions as to the question of chromosome-reduction, but it appears to me, from

<sup>1</sup> Fungi, Eng. Edit., p. 135.

<sup>2</sup> Die Entwicklung der Sexualorgane bei *Coleochaete pulvinata*. Flora, vol. lxxxv, 1898.



the few observations which I have been able to make, that a reduction in the number of chromosomes takes place during the mitosis in the oogonium and probably in the antheridium. Berlese states that in *C. Portulacae* the reduction takes place on the germination of the zygote; but Stevens says that in *C. Bliti* 'there is some evidence that makes it appear that there is a reduction in the number of chromosomes during the first mitosis' in the oogonium.

It is evident that further investigation is necessary before any definite statement can be made as to the reduction of chromosomes in the Peronosporae.

#### SUMMARY AND CONCLUSIONS.

1. The protoplasm of the oogonium becomes differentiated into a central vacuolate ooplasm and a granular homogeneous periplasm.

2. Immediately before this takes place a receptive papilla is formed on the oogonium at the place where the antheridium is in contact with it. At this spot the wall of the oogonium becomes very thin, and it is here that the fertilizing tube of the antheridium penetrates the oogonium.

3. The nuclei of the oogonium and antheridium undergo mitosis previous to fertilization.

4. Soon after the delimitation of the oosphere, a dense granular mass appears in the centre of it, which becomes converted into a homogeneous, ill-defined body, which stains more deeply than the surrounding cytoplasm. This *central body* appears to play some part in bringing the sexual nuclei together.

5. A single nucleus from the periplasm of the oosphere travels towards the central body and comes into close contact with it. The fertilizing tube of the antheridium also grows towards it and discharges a single nucleus, which comes into close contact with the female nucleus.

6. The sexual nuclei do not fuse at once, but remain apart for some time and increase in size, probably at the expense

of nutrient material in the cytoplasm. The male nucleus is at first slightly smaller than the female, but they soon become approximately of the same size and exhibit the same structure.

7. The fusion takes place while the nuclei are in the resting stage and not until the zygote is nearly ripe.

8. Before fusion takes place the central body disappears.

9. The ripe zygote is uninucleate. It contains a central large pale oil-globule and a peripheral layer of granular protoplasm, in which the nucleus is placed. The nucleus can be seen in the living condition as a pellucid spot in the protoplasm.

10. Of all the nuclei in the oogonium only one is set apart for the purpose of reproduction; the others remain in the periplasm and are used up to form the protective layer or layers of the oospore.

11. No difference is observable between the sexual nucleus and those that remain in the periplasm. They are the same size, have the same structure, and stain in a similar manner. It is probable therefore that all the nuclei of the oogonium are potentially sexual. In *C. Bliti*, as shown by Stevens, it appears to be a mere accident that determines which of the nuclei in the periplasm shall give off daughter-nuclei to the oosphere.

12. On comparing the various methods of fertilization and oospore-formation in the Peronosporaeae, we can, so far, distinguish three types. (1) Uninucleate oosphere, binuclear fusion and uninucleate oospore (*P. parasitica*). (2) Uninucleate oosphere, binuclear fusion and multinucleate oospore (*C. candidus*, *C. Portulacae* and *P. Ficariae*). (3) Multinucleate oosphere, multinuclear fusion in pairs and multinucleate oospore (*C. Bliti*). Whether *Pythium* will have to be regarded as a fourth type can only be decided by further investigation.

## EXPLANATION OF FIGURES IN PLATE XVI.

Illustrating Mr. Harold Wager's paper on *Peronospora parasitica*.

All the figures have been drawn with the aid of the camera lucida, and the apochromatic 2.0 m.m., 1.40 apert., of Zeiss, with ocular 8 ( $\times 1000$ ). The details were filled in with the aid of a magnification of 1500 (ocular 12), or 2250 (ocular 18).

Fig. 1. Young oogonium with antheridium and receptive papilla.

Fig. 2. Nuclei in the periplasm in process of division. The antheridium has put out a fertilizing tube, and the central body is just beginning to form in the ooplasm.

Fig. 3. Mitosis just completed; the central body is slightly more prominent.

Fig. 4. The central body is now very distinct, and a single nucleus from the periplasm is in contact with it. A nucleus is just about to pass from the antheridium into the fertilizing tube.

Fig. 5. The cytoplasm of the oosphere is finely vacuolate. A nucleus is seen in the fertilizing tube. There is now a distinct line of demarcation between the ooplasm and periplasm.

Fig. 6. A stage slightly later than Fig. 5, showing the first indication of the wall of the oosphere.

Fig. 7. Fertilizing tube nearly in contact with the female nucleus. It appears as if about to open at the apex. Two nuclei are to be seen in it.

Fig. 8. The sexual nuclei shortly after the entry of the male nucleus into the oosphere. The central body is already not so prominent and is beginning to disappear.

Fig. 9. Later stage: the nuclei are at some distance from one another. The wall of the oosphere is now very prominent.

Fig. 10. The sexual nuclei at a later stage. The apparent difference in size is due to the fact that one nucleus is seen from the end, the other from the side.

Fig. 11. The sexual nuclei in close contact. They are just beginning to fuse.

Fig. 12. The sexual nuclei in early stage of fusion. The cytoplasm contains large vacuoles.

Fig. 13. Later stage of fusion.

Fig. 14. Nuclei completely fused to form a single oval nucleus. The nucleoli are still visible.

Fig. 15. Fusion-nucleus, showing a distinct network and a single nucleolus.

Fig. 16. Nearly ripe oospore with single nucleus.

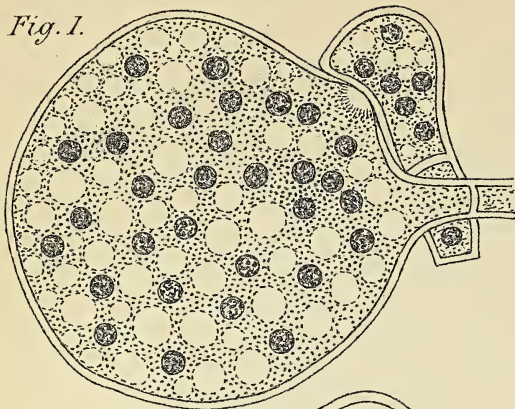
Fig. 17. Nearly ripe oospore with oogonial wall, showing how the periplasm is deposited on the wall of the oospore to form the exosporial layer.



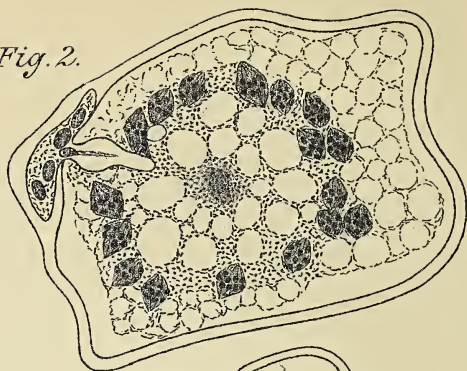




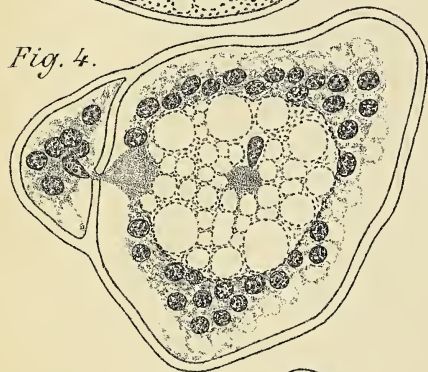
*Fig. 1.*



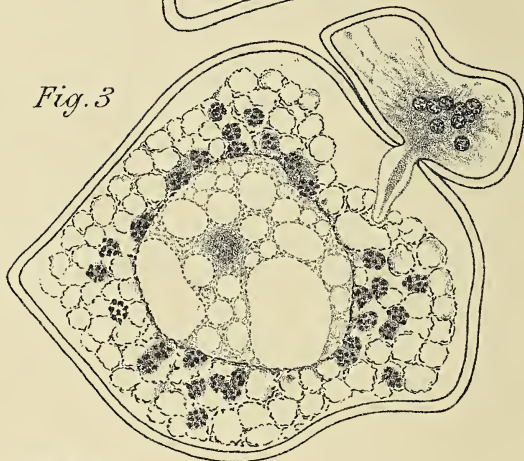
*Fig. 2.*



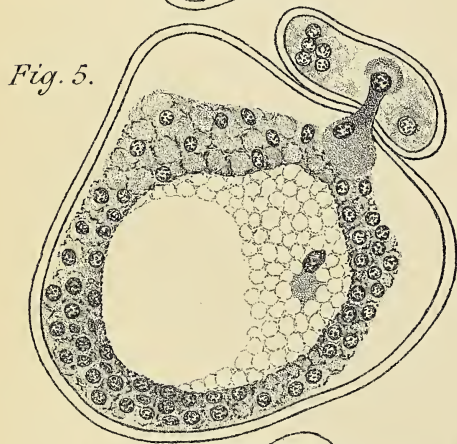
*Fig. 4.*



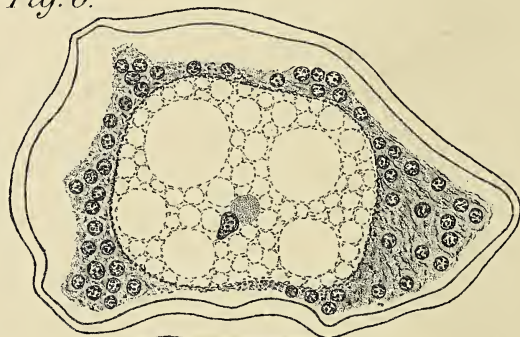
*Fig. 3.*



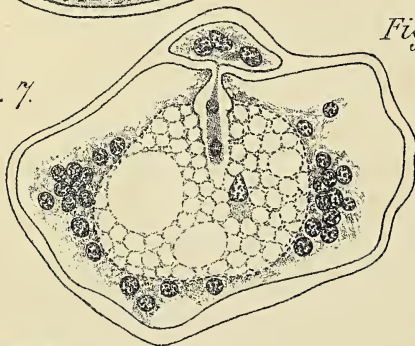
*Fig. 5.*



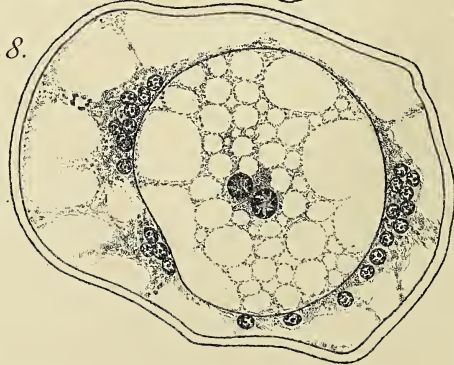
*Fig. 6.*



*Fig. 7.*



*Fig. 8.*



H. Waßer del.



Fig. 9.

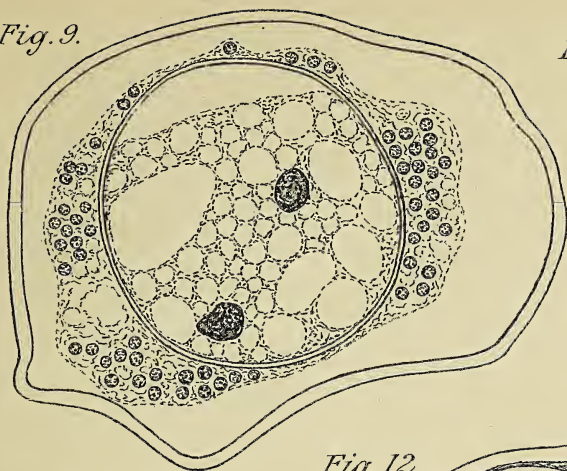


Fig. 10.

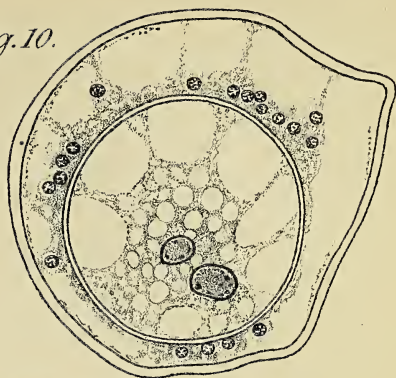


Fig. 11.

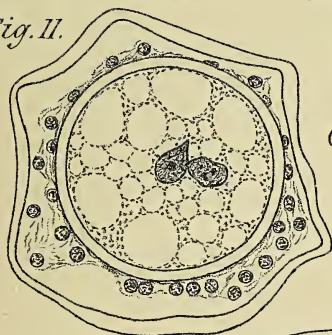


Fig. 12.

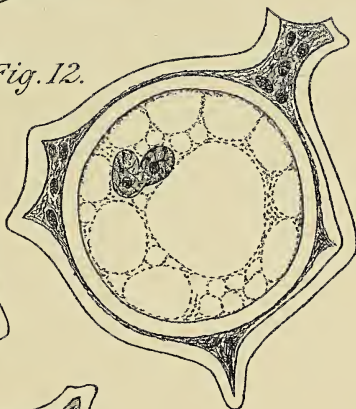


Fig. 17.

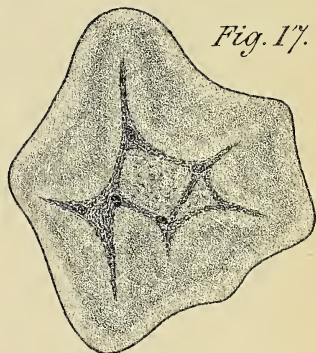


Fig. 13.

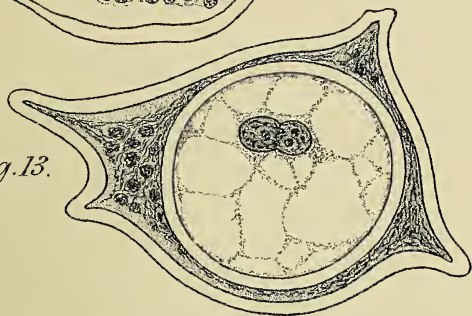


Fig. 14.

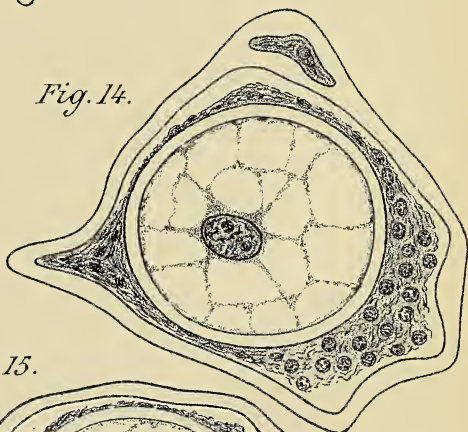


Fig. 16.

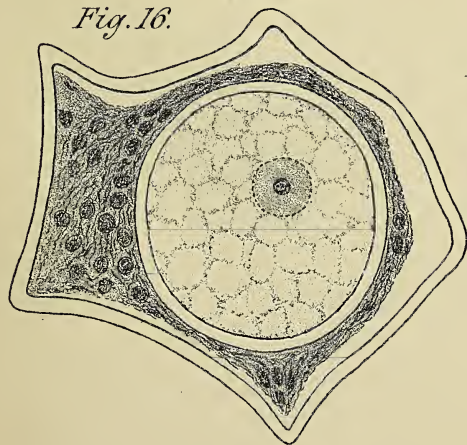
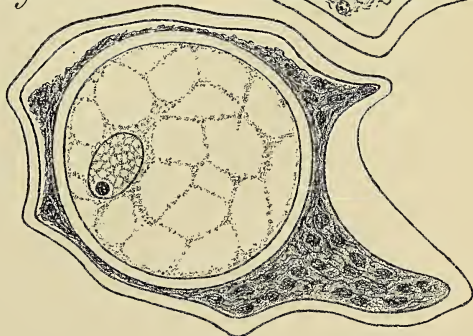


Fig. 15.





# Studies in the Development and Morphology of Cycadean Sporangia:

## II. The Ovule of *Stangeria paradoxa*.

BY

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Glasgow University.*

—♦—  
With Plates XVII and XVIII.  
—♦—

THE material, upon which the present investigation was made, was obtained from plants of *Stangeria paradoxa* growing in the Royal Botanic Gardens at Kew and Edinburgh. The cones, which served for the study of the development of the ovule, came from the Kew plant, while the fertilized ovules were the result of Mr. Harrow's successful artificial pollination of two cones, borne on a plant in the Edinburgh Gardens, with pollen from Kew. I am indebted to the Director of the Royal Gardens, Kew, and to Professor I. B. Balfour for placing the material at my disposal. Although the result of examination of the ovule and seed in this interesting genus of Cycads has been to show a general agreement with the corresponding structures in the genera already investigated<sup>1</sup>, the main stages will be briefly described.

<sup>1</sup> Cf. especially Warming, Oversigter d. k. dan. Vidensk. Selsk. Forhandl. 1877, 1879. Treub, Ann. Jard. Bot. Buitenzorg, 1885.



This will enable the development of certain parts of the Cycadean ovule, a detailed account of which is wanting, to be dealt with, and will afford a basis for the briefer description and general comparison of the other genera of recent Cycads, in a future number of these studies. The full consideration of the morphology of the Cycadean ovule must be deferred until further materials for this comparison have been accumulated, and the present paper will be restricted to a description of the phenomena observed in *Stangeria*, together with an estimate of the problem as illustrated by this genus.

The female sporophyll of *Stangeria* resembles the male sporophyll in general form. Two ovules are borne on each scale; they are situated one on either side of the short stalk, and their micropyles point towards the axis of the cone and slightly towards one another. The ovules are clearly not marginal, but each is situated slightly within the margin of a small area apparently corresponding to one of the soriferous areas of the lower surface of a male sporophyll. This will be evident on comparing Figs. 1 and 17, which represent sporophylls from the youngest and oldest cones investigated, with Fig. 1 of the first number of these studies<sup>1</sup>, representing the distribution of the sori on the young male sporophyll. The intra-marginal position of the ovule in this genus has been verified by the study of sections.

It was found to be impossible to obtain cones young enough to show the first stages of development of the ovule, without risk of injury to the plant. A median longitudinal section of one of the youngest ovules obtained is represented, from a photograph, in Fig. 2. Ovules at this stage of development are borne upon cones just visible among the bud-scales at the apex of the plant. As the figure shows, the rudiments of the free projecting portions of nucellus and integument are just recognizable. At this stage the ovule is only slightly constricted at its region of insertion on the sporophyll. In the median region of this stalk the cells are arranged in short

<sup>1</sup> Annals of Botany, vol. xi, p. 421, Pl. XXII.

vertical rows, which are not, however, strictly continuous with the rows of cells composing the nucellus. The latter is marked off from the integument by a series of flattened cells, which can be traced down from the place of separation of the free portions of integument and nucellus. Within this limit the nucellus is composed of vertical rows of cells, from five to seven of which are present in a median section. These rows can be traced to just below the epidermis, which is a definite layer of cells undergoing anticlinal divisions only. Although the continuity of the vertical cell-rows is usually clearly recognizable, a distinction is already apparent in the character of their component cells between the tip of the nucellus and an oval group of larger cells, which in older ovules becomes more sharply defined as the sporogenous tissue. About the centre of this sporogenous group a cell of larger size than the rest can be distinguished (Fig. 2). The nucleus of this cell, which is the mother-cell of the embryo-sac, is somewhat larger than those of its sister-cells. At this stage, however, the embryo-sac mother-cell has not become vacuolated. The position of the vascular bundles supplying the ovule is indicated at the base of the latter by desmogenstrands. As the ovule increases in size its different parts become more clearly defined from one another, and the projecting portions of the integument and nucellus-apex more prominent. The increase of the latter structure is due to periclinal divisions in the tissue just beneath the epidermis. The mother-cell of the embryo-sac, situated in the centre of the now clearly limited sporogenous tissue, has increased considerably in size and become vacuolated; it is still undivided, the large nucleus being situated towards the micropylar end of the cell, the wall of which has become thicker than the walls of the neighbouring cells. Fig. 3 represents the nucellus of an ovule at this stage.

The mother-cell of the embryo-sac in *Stangeria* undergoes considerable increase in size before dividing. The order of the divisions could not be accurately followed, but, as Fig. 4 shows, the mother-cell first divides into a larger lower and

a smaller upper segment ; other ovules have been seen with a row of three uncrushed cells (Fig. 5). From the comparison of these two stages, it would appear to be almost certain that it is the upper cell of the two resulting from the first division that divides again ; only the examination of the cells in the process of division can, however, settle this question definitely. In slightly older ovules the two cells of the row nearest to the micropylar end of the ovule are found crushed and flattened, the lowest cell, which is the young megaspore, being still uni-nucleate (Fig. 6). While these changes have taken place in the embryo-sac mother-cell, the ovule has undergone considerable alteration in size and form. This will be evident from Fig. 7, which represents the median longitudinal section of an ovule with the embryo-sac in the condition last described. As this figure shows, the indication of the division of the integument into three layers is becoming apparent. The margin of the integument has closed in somewhat, narrowing the micropyle, and the bluntly conical apex of the nucellus does not quite reach the level of the integument. The sporogenous mass is very distinct, and exhibits a difference between its more peripheral cells and those immediately around the embryo-sac. The cytological character, upon which this difference mainly depends, is, as the detailed drawing of the sporogenous tissue around the embryo-sac in Fig. 4 shows, the vacuolization of the more central cells. This is most marked in those which immediately surround the embryo-sac. These cells are also noteworthy for containing in their cytoplasm certain spherical bodies, the nature of which it has been impossible to determine with certainty from the material investigated. Since they also occur in the embryo-sac mother-cell it appears to be advisable to record their occurrence. Most frequently two of these spherical bodies are present in each cell ; they are situated in the cytoplasm at a greater or less distance from the nucleus, and stain similarly to the cytoplasm, though more deeply. As the figure shows, one of these bodies remains in each of the first two segments of the mother-cell, but it has not been



possible to demonstrate any connexion between them and the nuclear division.

None of the cones collected supplied the first stages of division within the megaspore, but from the study of the ovules next in age to the one last described (Fig. 7) it was clear that keeping pace with the increase in size of the ovule the embryo-sac enlarges. Its fairly thick wall is lined by a cytoplasmic layer in which numerous nuclei are distributed. Fig. 8 shows the longitudinal, and Fig. 9 the transverse, section of the nucellus of an ovule at this stage. The increase in size of the embryo-sac up to this stage does not appear to involve any considerable destruction of the sporogenous tissue, but to stand in relation to the growth of the ovule as a whole. As this takes place, the histological regions of the integument become more clearly marked out, and the differentiation of the xylem-elements takes place in the vascular bundles.

But the only point which calls for more special description has regard to the changes in form and mutual relations which take place in the nucellus and integument-margin in the region of the micropyle. In the youngest ovules examined the sides of the free apex of the nucellus are vertical, while its flat broad top stands on a level with the margin of the integument or even projects slightly above the latter (Figs. 2, 3, 10). In the general growth of the ovule the projecting portion of the nucellus keeps pace for some time with the free portion of the integument. It becomes more conical, however, but the flat top is retained. In the ovule represented in Fig. 11 this top is just below the level of the micropylar margin which is formed by the edge of the integument. As the latter comes to project further beyond the nucellus-apex, the micropyle is greatly narrowed, though the margin of the canal is still thin. In a slightly more advanced stage the integument becomes greatly thickened so that the micropyle forms a long passage, the external opening of which is narrow, while it widens out in passing inwards. Into this wider portion the conical tip of the nucellus-apex

projects, forming a very distinct pointed structure (Fig. 12). The distinctness of this is due partly to the increase in width of the nucellus-apex below the tip, but in part to the further growth of the tip itself, as is shown by the assumption of the pointed form. The ovule, when this stage of development of the nucellus-apex and micropyle has been reached, has an embryo-sac of considerable size, in which, however, no distinction of separate cells is apparent, the prothallus being still represented by a layer of cytoplasm with numerous nuclei. Little or no destruction of the sporogenous tissue has yet taken place.

No cones have been obtained illustrating the intermediate steps between such an ovule and the stage in which the embryo-sac has become completely filled with the tissue of the prothallus. The latter age is of special interest, since it probably is that at which pollination takes place in *Stangeria*. It will therefore be described in some detail, and at the same time the course of the vascular bundles in the ovule may be traced. The general relation and proportion of parts is shown in Fig. 13, which represents a median section of an ovule at this stage. In the integument the three layers are distinct, though the cell walls of the middle one are still unthickened. The long narrow micropylar canal is surrounded by the thick margin of the integument which projects as a short beak round the external opening. In the apex of the nucellus the pollen-chamber has been formed (Figs. 14, 15). The superficial cells of the pointed tip seen in Fig. 12 have their walls thickened, and form a very definite boundary to the sides of the chamber, suggesting a close comparison with the corresponding region of certain fossil Gymnospermous seeds<sup>1</sup>. At the actual apex of the conical projection, however, the epidermal cells have broken down, thus leaving an opening into the cavity formed by the disintegration of the internal tissue. From the base of this cavity a strand of more elongated cells

<sup>1</sup> See, for example, Brongniart, *Graines Fossiles Silicifiées*, Pl. III, Figs. 8 and 13 (*Cardiocrarpus augustodunensis*). Renault, *Structure comparée de quelques Tiges de la Flore Carbonifère*, Pl. XVII, Figs. 14 and 15 (*Cordaianthus Grand'Euryi*).

can be followed through the middle of the nucellus towards the embryo-sac.

No other disintegration or absorption of cells outside the sporogenous group has taken place. The sporogenous tissue, however, has undergone great changes with the increase in size of the megaspore. The latter is completely filled with the thin-walled parenchymatous tissue of the prothallus, the arrangement of the cells of which points to a gradual filling up of the cavity by divisions having taken place in a peripheral layer of cells. No starch is at this stage stored in the prothallus, which when fresh has a translucent appearance. Archegonia were not present, nor could the cells destined to form them be distinguished. The prothallus readily contracts during fixation, the wall of the megaspore which has become considerably thickened usually remaining more or less adherent to its surface. Around the megaspore a layer of cells was present which is clearly to be traced to the sporogenous group. The thick zone of sporogenous tissue present in earlier stages has, however, become reduced to a single layer. That this has taken place by the absorption of the cells between the outermost layer of sporogenous cells and the embryo-sac is indicated by the presence of smaller cells, in a more or less crushed condition, internal to the definite layer referred to. The latter appears thus to be derived from the most external layer of the sporogenous group, but material to follow the steps of the process was not available. The cells of this persistent layer (Fig. 16) are very large, and stand with the longer axis at right angles to the surface of the megaspore. Sometimes a single nucleus is present; often two are found in a cell. How long this layer of cells, which at this stage shows no signs of crushing or disintegration, persists, cannot at present be determined; in the fertilized seeds all trace of it was gone. Observations on aborting ovules showed that a similar increase in size of the outer layer of sporogenous cells takes place even when a normal embryo-sac is entirely wanting. In the light of the present facts it would appear to be a probable conclusion that, while



the majority of the sporogenous cells surrounding the embryo-sac simply become disintegrated and absorbed, the outermost form a more definite tapetal layer. This tapetum, while persisting longer than the more internal cells, ultimately disappears.

The course of the vascular bundles in the ovule is most easily followed at this stage, and may be briefly described. The general arrangement of the bundles in the sporophyll has been described and figured by Worsdell<sup>1</sup>. As he points out, the bundles passing to the ovule arise by division of a single branch from the vascular system of the lateral part of the sporophyll. This branch always divides into two bundles, one of which may again divide before reaching the base of the ovule. Thus two or three bundles of collateral structure enter the ovule. These bundles divide up at the base of the ovule into sixteen small bundles, eight of which bend out at once to form the vascular system of the outer layer of the integument, while the eight bundles alternating with them pass into the innermost layer. The bundles of both the inner and outer series, which thus take their origin at the same level, undergo subdivision though not to any great extent; those of the outer layer extend to the micropyle, while those of the inner series stop at the separation of nucellus from integument.

The remaining observations on the changes taking place in the ovule of *Stangeria* were made on two cones produced in the Edinburgh Botanic Gardens, and pollinated about the middle of February 1899. So far as can be judged, the cones at the date of pollination must have borne ovules of about the age of those last described. Dr. Balfour informs me that they were then erect on stiff peduncles, and considerably smaller than they became after pollination. The increase in size took place shortly after pollination, and was attended by a change in the position of the cones, which became pendulous. When the plant came under my observation in the beginning of July, the cone, excluding the peduncle, measured 14 cm. in

<sup>1</sup> *Annals of Botany*, vol. xii, p. 215, Pl. XVII, Fig. 5.

length, by more than 8 cm. in diameter at the widest part. At first, in the hope of obtaining a series of successive stages, one or two sporophylls were removed at intervals of a week or more. When, however, it was ascertained that even on July 4 embryos were present, this plan was abandoned and all the remaining ovules of one cone removed and fixed. The method is mentioned here, since, though useless in the present case, it would, if adopted early enough, enable all the main stages of fertilization and embryogeny to be studied on a single cone; the careful removal of sporophylls did not appear to injure the remainder of the cone in any way. Besides the seeds a number of aborting ovules were borne on the pollinated cone. As Fig. 17, which represents a scale bearing an aborted and a healthy ovule, shows, the former do not undergo the enlargement which normally succeeds pollination. The prothallus in the aborted ovules was either absent or represented by shrivelled remains, but the examination of these ovules was of use in affording pollen-tubes arrested at an earlier stage of their development than those in the seeds.

The seeds varied somewhat in size, but averaged about 2.5 cm. long by 2 cm. broad: the differences mainly depended on the variable thickness of the fleshy layer of the integument, which is especially thick at the micropylar end of the seed. Within the succulent layer, of a bright purplish pink colour, is the sclerotic layer, which is continued outwards as a pointed projection surrounding the inner fourth of the micropylar canal (Fig. 18). Within this is a brown layer of papery texture including the internal system of vascular bundles. Below the level of separation of nucellus and integument no distinction of these regions can be made in the internal layer. As Fig. 18 shows, the eight vascular bundles of the inner vascular system bifurcate once or twice but no anastomosis takes place. The remains of the nucellus form a thin cap over the micropylar end of the prothallus. In the centre of this, the pollen-chamber with its conical apex is to be seen. The prothallus, now of a white colour owing to its cells being

filled with starch, is still enclosed within the wall of the megaspore. Just beneath the pollen-chamber its surface is depressed, to form a single cylindrical cavity about 2 mm. in diameter with vertical or slightly overhanging sides. At the base of this archegonial depression (Fig. 19) the necks of from two to four archegonia were distinguishable.

Only a single unfertilized archegonium was met with ; it is represented in Fig. 20. From this the close agreement between *Stangeria* and other Cycads, as regards the structure of the archegonium, will be evident. The neck consists of two cells projecting above the general level of the surface of the prothallus, and the cap-like ventral canal-cell bears much the same relation to the large ovum as in *Cycas*. The nucleus of the ovum was situated towards the upper end, while in a central position a small irregular area of less granular cytoplasm was to be seen. The thick wall of the ovum is strongly pitted, several pits being present opposite each cell of the layer of the prothallus, forming the wall of the venter. Sometimes the pair of neck-cells was met with, although the lower cell had not developed into an ovum ; such a case is represented in Fig. 21. The structure of the prothallus does not call for special remark ; its cells, with the exception of those around the archegonia, and the limiting layer, which presents the structure described by Warming<sup>1</sup> in *Zamia*, being filled with starch.

Having considered the general structure of the pollinated ovule of *Stangeria*, there remain, for more detailed consideration, two classes of facts, those relating to the changes in the pollen-chamber after pollination, the germination of the pollen-grain, and the mode of fertilization, and those relating to the development of the embryo. On both of these questions, although the material did not afford a complete series of stages, it has been found possible to draw sufficient conclusions to bring *Stangeria* into relation with those Cycads in which the phenomena of fertilization and embryogeny have been fully studied.

<sup>1</sup> Warming, loc. cit., 1877. Pl. III, Fig. 30.



## II. The Ovule of *Stangeria paradoxa*. 291

When pollination takes place the ovule is probably in the condition represented in Fig. 13. In normal ovules, as growth proceeds, the nucellus becomes reduced to the pollen-chamber in the centre of a thin cap of compressed tissue; the stages in the absorption of tissue leading to this were not observed, the nucellus of the aborted ovules on the pollinated cone being in the former state, and that of the seeds in the latter. Pollen-grains were present in all the pollen-chambers. While some of the grains presented no alteration or were merely attached to the wall of the chamber by short tubes, others had undergone great changes. The pollen-tubes had penetrated the tissue of the nucellus, and were found radiating on all sides from the pollen-chamber (Figs. 22, 23). Usually their course lay just beneath the surface of the nucellus, but in some of the aborting ovules, in which the more internal tissue of the nucellus was not absorbed, the tube had grown inwards through this; this latter course was evidently abnormal. The pollen-tubes were of considerable length, and of a much greater diameter than the pollen-grain itself. The free portion of the tube to which the wall of the pollen-grain was attached bent downwards towards the base of the pollen-chamber, which, by the absorption of the internal tissue of the nucellus, offers a free passage towards the megaspore (Fig. 24). The free end of the tube did not appear to project much beyond the lower opening of the pollen-chamber, and was thus in the seed separated from the archegonium-neck by almost the whole depth of the archegonial depression. To what extent this depression is fully formed at the time of fertilization it is impossible to say, but there is sufficient evidence to show that the passage from the pollen-tube to the archegonium-neck is effected by the independent motility of the spermatozoid. The details of the development of the latter from the generative cell of the pollen-grain could not be followed, but in the free ends of pollen-tubes in the aborted ovules two fully developed spermatozoids were present. These had apparently died in the unopened pollen-tubes, and consequently stained very badly, but the spiral cilium-bearing

band was rendered prominent by the use of Heidenhain's Haematoxylin (Fig. 25). In the pollen-chambers of the seeds the free ends of the pollen-tubes were always open and empty (Fig. 23). As the vertical section of the chamber shows (Fig. 24), the spermatozoids could easily find their way into the archegonial depression, and in fact remains of dead spermatozoids were not unfrequently met with against the wall of the latter; they were usually entangled in an amorphous substance which could be traced into the necks of fertilized archegonia. Analogy would appear to justify the conclusion that the archegonial depression had for a time been filled with fluid, in which the motion of the spermatozoids had taken place; this had, however, disappeared at the time of collecting the material. More or less disorganized spermatozoids, which had failed to effect fertilization, were also found just within the archegonium.

In the cytoplasm of the ovum, at the end adjoining the archegonium-neck, remains of the spermatozoid, by which fertilization had been effected, were frequently visible. The persistent part was the spiral cilia-bearing band; it was usually so altered in form as to give no idea of the shape of the spermatozoid, though the cilia attached to it were visible. Fig. 26, however, shows one of the sections through such a spiral band, the form of which has been retained; reconstruction of the series showed that it formed a conical spiral of five turns.

These observations taken together show clearly that the behaviour of the pollen-grain in the pollen-chamber of *Stangeria*, the production of spermatozoids, and the mode of entrance of the latter into the archegonium, agree with what has been described for *Cycas*<sup>1</sup> and *Zamia*<sup>2</sup>. Further, although well-fixed spermatozoids were not available for study, the form of the spiral band (blepharoplast) leaves no room for doubt that they were of the same type as those of the genera mentioned.

<sup>1</sup> Ikeno, Jahrb. für wiss. Bot. xxxii, p. 557.

<sup>2</sup> Webber, Botanical Gazette, xxiv, p. 16.

The actual process of fertilization was not observed, the youngest fertilized archegonium showing several nuclei in its cytoplasm. These were situated in the central area of more finely granular protoplasm referred to in the description of the archegonium, and, in the only embryo of this age found, were undergoing karyokinetic division. Slightly older embryos (Fig. 27) had numerous small nuclei in the cytoplasm; these tended to take up their position at the periphery, and were most numerous at the lower end of the embryo. In the next stage, though the embryo is still enclosed in the wall of the archegonium, it no longer forms a continuous mass filling the venter of the latter, but appears as a hollow sac enclosing a large cavity (Fig. 28). The occurrence of this change has been described by Treub<sup>1</sup> and Ikeno<sup>2</sup> in the similar embryo of *Cycas*; in this genus also it would appear to take place before the embryo has undergone any increase in size. As in *Cycas*, the embryo with its suspensor is developed entirely from the lower end of the hollow sac of tissue. The upper portion remains as a thin layer of cytoplasm with free nuclei in it, lining the thin cell-wall, with which the embryo surrounds itself. The cells forming the thicker lower end of the sac become enclosed by cell-walls, and, as Fig. 29 shows, become differentiated into the suspensor and embryo. The latter has broken through the wall of the archegonium, and projects into the tissue of the prothallus. At this stage the embryos from the different archegonia of a prothallus, though inclined towards one another, are separated by tissue of the prothallus. The short suspensor is composed of vacuolated cells; those forming the embryo are meristematic, and can be distinguished into an epidermal layer and the internal smaller-celled tissue. As the suspensors of the several embryos increase in length, the latter come to occupy a single cavity, formed by the progressive destruction of the surrounding tissue of the prothallus. In the upper part of this common space the suspensors, which become considerably coiled and folded, are found, while at the base, embedded in the still

<sup>1</sup> Ann. Jard. Bot. Buitenzorg, iv, p. 5.

<sup>2</sup> loc. cit.



coherent tissue, are the embryos (Fig. 30). The embryo is usually wedge-shaped, but exhibits no greater differentiation of its tissues than in the earlier stage. One of the embryos has, as Fig. 30 shows, by this time become larger than the others, and penetrates more deeply into the prothallus. This isolation of the successful embryo is more apparent in Fig. 31, which represents the largest embryo found in an unsown seed. In the process of elongation of the suspensor its base is often forced upwards towards the neck of the archegonium, the contents of which have, as a rule, by this time entirely disappeared.

The embryo in one seed, which had been placed under suitable conditions for germination in the Edinburgh Gardens, was examined after some weeks. As Fig. 32 shows, it was not firmly embedded in the prothallus, but hung free on the suspensor into the cavity formed by the destruction of the surrounding tissue. The embryo had increased somewhat in size, but showed no advance in morphological differentiation, no indication of the cotyledons being apparent: the tissue composing it resembles an apical meristem more closely than it did at earlier stages.

In the next stage obtained the primary root of the seedling was already over an inch in length. The seedling resembled those of other Cycads at this stage. The anatomy agreed generally with the description by Worsdell<sup>1</sup>, founded on a somewhat more advanced seedling of *Stangeria*. It would appear to be a justifiable inference from a comparison of the embryos figured in Figs. 31 and 32 with the seedling, that the intimate connexion, which ultimately obtains between cotyledons and prothallus, is effected by the origin of the former on an embryo hanging free in the absorption cavity. This explains the way in which the stem-apex is freed from contact with the prothallus, and is in a position to be carried outside the seed by intercalary growth of the cotyledons when germination takes place.

One or two deviations from the normal embryogeny may

<sup>1</sup> Journ. Linn. Soc., vol. xxxiii, p. 447, 1898.

be mentioned in conclusion. Evidence was obtained that small accessory embryos<sup>1</sup> may be formed on suspensors which terminate in normal embryos, but the material did not permit of their detailed study. In one instance an embryo was found to have originated at the upper end of the archegonium. The suspensor, which was of considerable length, projected from the archegonium-neck into the depression at the micropylar end of the prothallus (Fig. 33), but the embryo borne on it had apparently disappeared. Probably the disappearance of the fluid filling the archegonial depression at the time of fertilization had arrested its growth. A small accessory embryo was present within the archegonium.

The above description will have made evident the close general agreement in development of ovule, pollination, fertilization, and embryogeny, which *Stangeria* presents with other genera of Cycads. The phenomena observed in *Stangeria* may be briefly summarized:—

1. Two ovules are developed on each sporophyll; they are situated one on either side of the short stalk and are not marginal, but borne on the morphologically lower surface.

2. The development of the ovule is similar to that of *Ceratozamia*, save that the mother-cell of the embryo-sac appears to attain a greater size before it undergoes division into three. The parts of the ovule (stalk, integument, nucellus, sporogenous group and megaspore) bear the same relations to one another as in *Ceratozamia*.

3. At the time of pollination the prothallus fills the megaspore, but archegonia are probably not present; the sporogenous tissue is represented by a single persistent layer, possibly tapetal in nature; the pollen-chamber is fully formed, but absorption of the tissue of the nucellus between it and the megaspore has not commenced.

4. The pollen-tubes penetrate the nucellus as in *Cycas* and *Zamia*; in the free end of each two spermatozoids are formed.

<sup>1</sup> Such accessory embryos are described in *Macrozamia* by Miquel, Nouveaux Matériaux pour la Connaissance des Cycadées, p. 18, Pl. II, figs. 4 and 8 (Arch. Néerland., t. iii, 1868).

The spermatozoid is of large size and possesses cilia attached to a blepharoplast which forms a spiral of five turns.

5. By the absorption of the intervening tissue of the nucellus a free passage is formed between the pollen-chamber and the prothallus. The spermatozoids, on their escape from the pollen-tube, which only bends down for a short distance, probably reach the archegonium by their independent motility.

6. The embryos, which are formed singly at the lower ends of the archegonia as in *Cycas*, possess long suspensors and come to occupy a common cavity formed by absorption of the tissue of the prothallus; ultimately one embryo becomes dominant in each seed.

7. The embryo frees itself from the prothallus before the cotyledons are formed; these become in turn intimately attached to the prothallus.

#### THE MATURE MICROSPORANGIUM OF STANGERIA.

##### A CORRECTION.

In the first number of these studies too close a comparison was drawn between the microsporangia of *Stangeria* and the sporangia of *Angiopteris*. Since the resemblance between the microsporangium and ovule may be of importance in discussing the results obtained from the study of *Stangeria*, this opportunity may be taken of correcting the erroneous impression conveyed in my earlier paper; at the same time some additional facts ascertained regarding the microsporangium may be stated.

The error referred to does not concern any of the points illustrated in the figures of my former paper, but is contained in the reference to the sporangium of *Angiopteris* on p. 432. The view there expressed was that bands of thicker-walled epidermal cells on either side the line of dehiscence in the microsporangium of *Stangeria* might be regarded as corresponding to the distinct lateral bands of indurated cells in a corresponding position in *Angiopteris*. Further observa-



tions have led to the conclusion that the recognition of these as definite bands in *Stangeria* was due to the sporangial wall not being quite mature, and that, although the epidermis in this position and extending down from the apex of the sporangium towards the lower surface has somewhat thicker walls than that on the sides of the sporangium, the differences do not warrant the close comparison made with any particular sporangium among Ferns, such as that of *Angiopteris*.

With regard to the group of small isodiametrical cells situated at the apex of the sporangium, these further observations have shown a considerable range of variation in *Stangeria*. As a rule the group consists of only a few cells with fairly thick colourless walls, surrounded by the brown thick-walled cells, which present no distinctive characters when they border on the apical group. It is this apical group which has usually been compared to the annulus of *Osmunda* and *Angiopteris*. But some of the microsporangia of *Stangeria* were found to present an apical group of larger size composed of fairly thin-walled cells. Around such a group as this the thicker walled brown cells form a definite and regular margin. Further, the series of cells immediately adjoining the apical group in these cases is characterized by its definiteness, and suggests a comparison with the annulus of the *Schizaeaceae*, in its less defined form, as seen in *Mohria*. It is not intended to imply by this comparison any actual relationship with the *Schizaeaceae*, but to suggest that, until more evidence is forthcoming as to the type of sporangium from which the Cycad microsporangia are derived, the nature of the apical group must be regarded as an open question. On the one hand, as is usually assumed, it might correspond to the annulus, on the other it may be more nearly comparable to the region of the sporangial wall above the annulus in the Schizaeaceous sporangium. It may be hoped that a comparative study of the other genera of Cycadaceae will throw further light on its true nature.

## THEORETICAL DISCUSSION OF RESULTS.

In this and the preceding number of these studies, the development and structure of the microsporangium and ovule of *Stangeria* have been examined, so far as was possible on material obtained from cultivated plants. The general agreement of the results attained for this genus with those previously recorded by Warming and Treub, renders a similarly detailed investigation of the remaining genera unnecessary. It therefore appears advisable to discuss briefly the general conclusions indicated by the facts in connexion with *Stangeria* and to reserve for a future number of these studies a comparative treatment of the sporangia of the other Cycads; this will further enable the general view here advanced to be tested in detail. The equally important data supplied by the vegetative organs of the *Cycadaceae*, and the evidence regarding the phylogeny of the group obtainable from fossil plants, will also be most suitably considered after the series of existing genera has been comparatively treated. In this place the consideration of the general phylogenetic problem will be left on one side and only the morphology of the sporangia discussed. It is only necessary to point out here the justification, which the geological history of the seed-bearing plants affords, for considering the nature of the Cycadean ovule and its relation to the microsporangium, without extending the comparison to the other Gymnosperms or to the Angiosperms. So far as is at present known, Cycadean plants can be traced back to the Permo-Carboniferous flora. In rocks of this age well-preserved seeds are also found, some of which are probably Cycadean, while others may belong to the Cordaiteae. The remains of the latter group carry the first origin of the Cycadeo-Cordaitan type of ovule to beyond the earliest period of which adequate fossils remains are preserved. It is unnecessary for our present purpose to consider the probable degree and kind of relationship between Cordaiteae and Cycadaceae.

## II. The Ovule of *Stangeria paradoxa*. 299

The general type of ovule is the same and may fairly be regarded as the most primitive preserved among existing plants, a conclusion that has received the strongest support from the discovery in recent years of the peculiar behaviour of the pollen-grain, and the free passage of motile spermatozoids from the pollen-chamber to the archegonium in Cycads. It thus appears to be a legitimate course to pursue, to seek for indications of the probable mode of origin of the Cycadean ovule from the living forms, in which alone the ontogeny is accessible, since the group has preserved so many primitive characters apparently little altered.

The inquiry, as limited to the sporangia of *Cycadaceae*, has two aspects, the question as to the group of Pteridophyta from which the Cycads were derived, and that of the morphology of the Cycadean ovule. The former of these questions, if capable of a satisfactory answer, would afford a starting-point for the solution of the latter, by indicating the type of sporangium, from which both microsporangium and ovule were derived, though it would remain a question whether the ovule is to be regarded simply as a modified sporangium, or as a sporangium together with part of the sporophyll. Looking to the habit, structure, and floral morphology of both *Cordaiteae* and *Cycadaceae* it cannot be said that their relationship to any particular group of Vascular Cryptogams is at present satisfactorily proved, but as regards the *Cycadaceae* a number of facts are known pointing to an origin from a Fern-like group of plants. This (which may be taken as the most probable hypothesis open to us at present) would accord well with the position and arrangement of the microsporangia.

Even if this question is regarded as in some degree an open one, it is possible to inquire into the morphology of the Cycadean ovule, since we may assume with some probability that the microsporangium, with its general resemblance to the more bulky sporangia found among the Ferns, has not come to differ very widely from the sporangia of the unknown ancestral group.

Before, however, considering the facts regarding *Stangeria*



from this point of view, the main views recently expressed on the morphology of the Cycadean ovule by investigators who have studied the group must be shortly summarized. Since 1880, when the sporangium was definitely recognized by Goebel as an organ *sui generis*, the comparison between the ovule and a bud has been practically abandoned. The nucellus has been clearly recognized as corresponding to a sporangium; the integument being regarded either as homologous with such an indusium as that of the *Hymenophyllaceae*<sup>1</sup>, or simply as a new formation. In his earlier work Strasburger regarded the whole ovule as corresponding to a sporangium, but in his latest statement<sup>2</sup> on the Gymnospermous ovule he definitely compares the nucellus with the sporangium, and adopts the view that the integument is a new formation. One point common to the estimate of the Cycadean ovule formed by both these observers and to the conclusion of Treub<sup>3</sup>, is the comparison of the ovule in this group with a sporangium enclosed within the tissues of the sporophyll, and not projecting freely from it, such, for instance, as the individual sporangia in the *Ophioglossum* spike. In *Ceratozamia*, the ovule of which he investigated, Treub recognized the appearance of the sporogenous group of cells *within the sporangiferous lobe of the sporophyll*, and regards as a new formation the free upgrowth of nucellus and integument from the summit of the sporangiferous lobe turned towards the axis of the cone.

In these views of the nature of the Cycadean ovule and the type of sporangium from which it may be derived, there is, so far as I am aware, no comparison instituted between the microsporangium and ovule, except as regards the sporogenous group and the layers immediately surrounding it. Indeed the microsporangia and the ovules of Cycads have usually been compared with such very different sporangia as those of *Angiopteris* and *Ophioglossum* respectively. It is probable that this absence of direct comparison between the

<sup>1</sup> Warming, *Syst. Bot.*, p. 169.

<sup>2</sup> Bot. Practicum, 3rd ed., p. 514.

<sup>3</sup> loc. cit., 1885, p. 48.

Cycadean ovules and the microsporangia has been due to the ovules investigated having a marginal position on the sporophyll, while the microsporangia are usually borne on the under surface. In *Stangeria* (and the case is the same with some other genera) the ovule is clearly intramarginal, and careful comparison of the male and female sporophylls shows that as regards position the ovule clearly corresponds to a microsporangium or to a sorus of microsporangia. In seeking for evidence as to the homology of any structure, relative position, although important, must be confirmed by the study of the mode of development and by the structure of the organ when mature. With regard to the development considerable correspondence between the ovule and the sorus can be recognized in the early stages. The first stages of the ovules of *Ceratozamia* described by Treub, from which there is no reason to assume *Stangeria* to differ, show clearly that the sporogenous group of tissue has the same origin as in the microsporangium, though probably in the case of the ovule derived from more numerous hypodermal cells. Even in the ovule represented in Fig. 2 the continuity of the rows from the hypodermal layer to the base of the sporogenous group may be traced. The differences between the development of the sorus of microsporangia and the ovule only become pronounced when the arrest of growth in the region between the individual sporangia takes place, or (a mode of expressing the same fact which is probably more correct) when active growth becomes localized around each archesporial group. The comparison at later stages must be made between the individual sporangium and the ovule; in both a large oval mass of sporogenous tissue is developed, which is marked off more clearly by the flattening of the layers of cells immediately around, and readily separates at this region. Further, the comparison is perhaps justifiable between the persistent tapetal layer, derived from the outer layer of the sporogenous tissue in the microsporangium, and the layer which has the same place of origin in the ovule. The facts of development then would support the view that the ovule

of *Stangeria* is homologous with a sorus of microsporangia, in which only one sporangium is present, so that the sorus as a whole had kept pace with the developing sporogenous mass. When the origin of the microsporangium in the sorus is taken into account, it is evident that there is little, if any, difference between this conclusion and the statement that the ovule is homologous with a microsporangium. It is not difficult to recognize the equivalent of the projecting tip of the nucellus in the divisions which take place in the wall of the microsporangium above the sporogenous tissue. These divisions are better represented in the microsporangium of *Zamia* than in that of *Stangeria*; in the former case, as Treub's figures<sup>1</sup> show, they lead to the development of a pointed tip to the sporangium, which presents a striking resemblance to the apex of the nucellus of the ovule. There remains only the integument to be considered. The projecting portion of this is clearly unrepresented in the microsporangium, and, on the view here suggested, would be regarded as an annular upgrowth, around the apex of the nucellus, of the bulky sporangial wall, or, which comes to the same thing, of the edge of the receptacle which had kept pace with the single sporangium. It will be evident from this comparison of the ovule with the microsporangium, and from the mode of origin of the integument suggested as probable, that the ovule is not regarded as a sporangium sunken in a lobe of the sporophyll. The facts appear to the author more naturally explained, when we compare the ovule to a sorus consisting of a single sporangium, which develops on the whole similarly to a microsporangium, save that it is bulkier, and the wall from the first thicker.

If the more advanced ovule, say at the period of pollination, be compared with the mature microsporangium, it is not surprising to find nearly all traces of the structure of the epidermal layer, adapted for dehiscence in the microsporangium, absent in the ovule. It may, however, be pointed out that in the Cycadean type of ovule we have a mega-

<sup>1</sup> Treub, loc cit., 1885, Pl. II, Fig. 4.



sporangium which still opens to the outside, so that free access is given to the spermatozoids to reach the megaspore though the latter is not liberated. As regards the structure of the opening, it can only be tentatively suggested that the pollen-chamber, with its thick-walled cells, may correspond to the ring of thick-walled cells around the apical cap of the microsporangium.

Whatever the modifications were that led to the origin of the Cycadean type of ovule, no direct evidence of their nature is available at present, and it is thus necessary to connect the facts by as reasonable an hypothesis as possible. It may be pointed out, however, that the view above expressed, which rests primarily on the similar position held by the ovule of *Stangeria* to that of one of the sori of microsporangia, derives support from an examination of the relation borne by the individual sporangium to the sorus in some groups of Vascular Cryptogams. The reduction of the number of sporangia in the sorus to a single one occurs not infrequently in some species of *Gleichenia* and is the normal state of affairs in the majority of the *Schizaeaceae*, in which the monangial sorus has been definitely recognized. In these cases the variation is unconnected with heterospory, but its connexion with this is strikingly illustrated by *Azolla* among the *Hydropterideae*. It will be obvious that the reduction in number of the sporangia in a sorus, and along with this of sori on a sporophyll, would be still more necessary when the completion of the changes within the megaspore and the production of the embryo were all to go on at the expense of the parent plant. There would thus appear to be, on *a priori* grounds, good reason to assume that the need of such a reduction in number of sporangia in the sorus would have been even greater in the evolution of the ovule of Cycads than in the group of *Hydropterideae*, which are here cited as an unrelated but somewhat parallel case.

A provisional examination of material of a number of other genera of Cycads has not disclosed anything inconsistent with the view of the nature of the Cycadean ovule here put

forward, while indications are not wanting that the comparative study of these genera will afford it further support. As, however, the material collected does not as yet allow of a sufficiently wide comparison, this will be deferred for the present. Until this is done, however, the view that the Cycadean ovule is the equivalent of the male sorus can only be put forward as a provisional statement, which will have to be carefully tested by a comparative examination of the whole series of living Cycads, in the light of the evidence obtainable from extinct forms.

## EXPLANATION OF FIGURES IN PLATES XVII AND XVIII.

Illustrating Dr. Lang's paper on the ovule of *Stangeria paradoxa*.

### PLATE XVII.

Fig. 1. Young sporophyll, bearing two ovules, viewed from its point of attachment to the axis; the ovules are situated within the margin on the abaxial surface. *st*=cut surface of stalk of sporophyll. ( $\times 25$ .)

Fig. 2. Median longitudinal section of a young ovule; the free portions of nucellus and integument are just recognizable, and the mother-cell of the embryo-sac can be distinguished in the centre of the sporogenous tissue. ( $\times 110$ .)

Fig. 3. Similar section of a slightly older ovule, the integument being omitted; the mother-cell of the embryo-sac has increased in size and become thicker-walled but is still undivided; the sporogenous tissue is shaded. ( $\times 375$ .)

Fig. 4. Mother-cell of embryo-sac divided into two segments, with the adjoining sporogenous tissue; in the cells of the latter the spherical bodies described in the text are visible. ( $\times 375$ .)

Fig. 5. Mother-cell of embryo-sac divided into three segments, the lowest of which is destined to become the embryo-sac. ( $\times 375$ .)

Fig. 6. Young embryo-sac, still uni-nucleate, with the two sister-cells in a crushed condition at its upper end. ( $\times 375$ .)

## II. The Ovule of *Stangeria paradoxa*. 305

Fig. 7. General view, in median longitudinal section, of the whole ovule at the stage with uni-nucleate embryo-sac. ( $\times 50$ .)

Fig. 8. Longitudinal section of nucellus of older ovule, showing the embryo-sac with numerous free nuclei in its cytoplasm. ( $\times 70$ .)

Fig. 9. Transverse section of ovule of similar age passing through the sporogenous tissue and megaspore. ( $\times 70$ .)

Figs. 10-12. Series of photographs of the apex of the nucellus and micropyle from ovules of increasing age; in Fig. 12 the pointed projection of the nucellus destined to form the pollen-chamber is just apparent. ( $\times 110$ .)

Fig. 13. Median longitudinal section of older ovule; the sporogenous tissue is reduced to a single layer around the megaspore, which is filled with the prothallus; the pollen-chamber is fully formed. ( $\times 7$ .)

Fig. 14. Micropylar region, from a similar section, showing the relation of the pollen-chamber to the micropyle. ( $\times 25$ .)

Fig. 15. Pollen-chamber from a similar section of an aborting ovule, showing the large thick-walled cells forming the wall. ( $\times 70$ .)

Fig. 16. Part of a section through an ovule of the same age as that in Fig. 14. From left to right are seen the edge of the prothallus, the wall of the megaspore, the persistent layer of the sporogenous tissue, and the tissue of the integument. ( $\times 110$ .)

### PLATE XVIII.

Fig. 17. Sporophyll from the fertilized cone bearing a seed and an aborted ovule; seen from above. (Natural size.)

Fig. 18. Dissected seed, showing the succulent and woody layer of the seed-coat cut through, the inner layer with its system of vascular bundles covering the prothallus, and the remains of the nucellus on the micropylar end of the latter. (Natural size.)

Fig. 19. View of the micropylar end of the prothallus from above, showing the apical depression with the necks of four archegonia at its base. ( $\times 2$ .)

Fig. 20. Longitudinal section through an unfertilized archegonium. ( $\times 25$ .)

Fig. 21. Archegonium-neck from the base of the archegonial depression, probably representing an arrested archegonium. ( $\times 100$ .)

Fig. 22. Surface view of the pollen-chamber and remains of the nucellus from a seed, showing the pollen tubes radiating out from the chamber. ( $\times 7$ .)

Fig. 23. One half of a similar specimen viewed from the inside, showing the blind ends of the pollen-tubes. ( $\times 7$ .)

Fig. 24. Median vertical section through the pollen-chamber and nucellus of a seed. ( $\times 35$ .)

Fig. 25. Part of a vertical section through the pollen-chamber of an absorbed ovule showing a dead spermatozoid (spm) in a pollen-tube which has been cut obliquely. ( $\times 110$ .)

Fig. 26. Spiral body of spermatozoid embedded in the upper part of the cytoplasm of a young embryo. ( $\times 110$ .)

Fig. 27. Young embryo showing commencement of vacuolization. ( $\times 25$ .)

Fig. 28. Older embryo still enclosed in the archegonium-wall, showing the large central cavity. ( $\times 25$ .)



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Fig. 29. Longitudinal section of embryo borne on a short suspensor in the tissue of the prothallus; the archegonium-wall is broken through at its lower end. ( $\times 70$ .)

Fig. 30. Older embryo on long suspensor, the embryo still firmly fixed in the prothallus-tissue. ( $\times 70$ .)

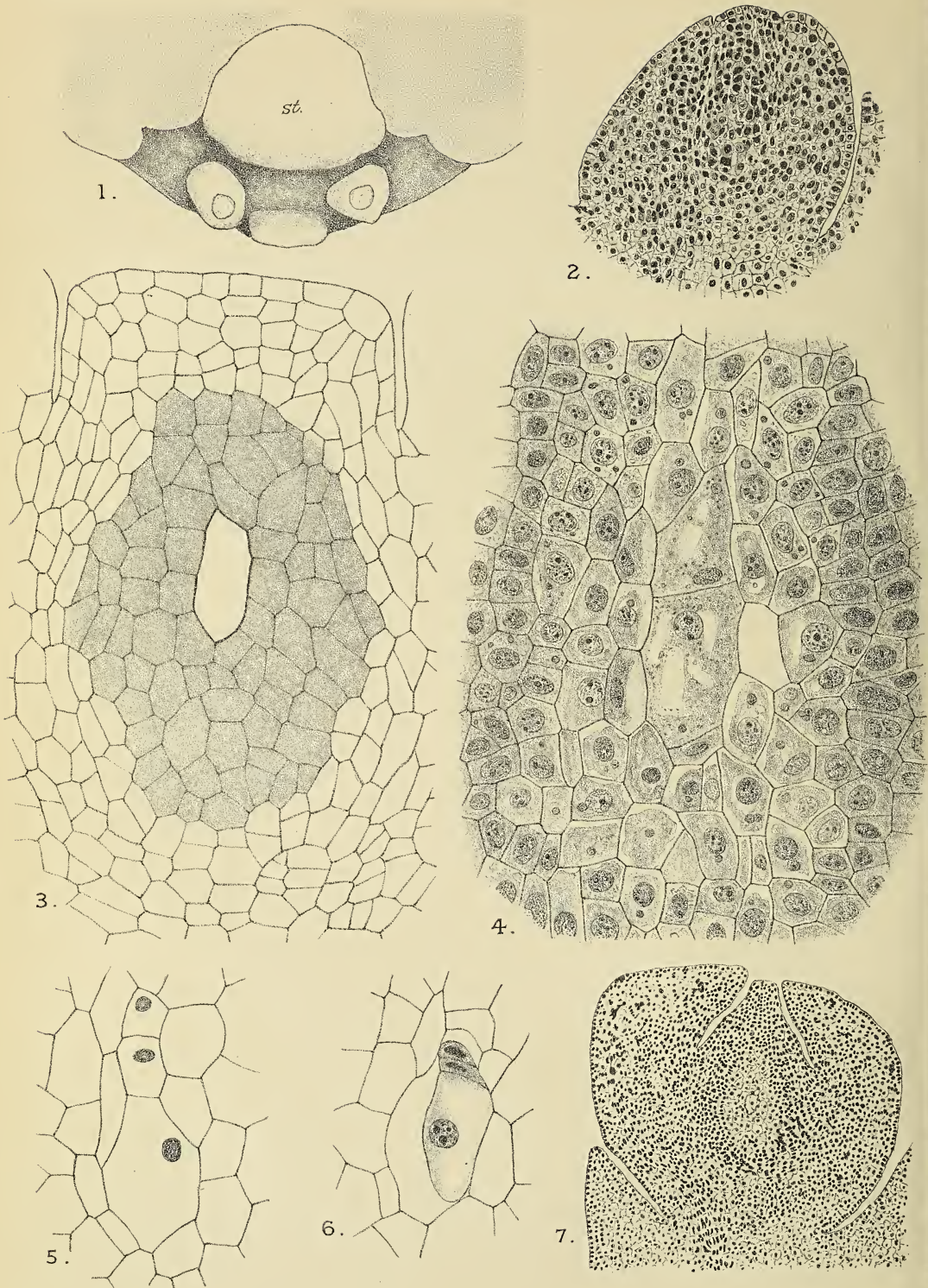
Fig. 31. Similar section of the largest embryo found in a seed still borne on the cone; the embryo shown has outstripped its competitors more completely than that in Fig. 30. ( $\times 70$ .)

Fig. 32. Similar section of an embryo from a seed sown for some weeks, the embryo is at this stage suspended freely in the cavity formed by the destruction of the tissue of the prothallus. ( $\times 70$ .)

Fig. 33. Abnormal embryo, the suspensor having grown through the archegonium-neck into the archegonial depression; the remains of a small accessory embryo are seen below the base of the suspensor. ( $\times 50$ .)

Figs. 1, 3-6, 13, 18, 19, 21-24 and 28 from drawings, the rest from photographs.





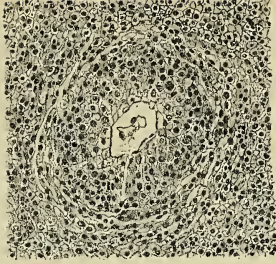
W. H. Lang, del. et photo.

LANG. — STANGERIA PARADOXA.





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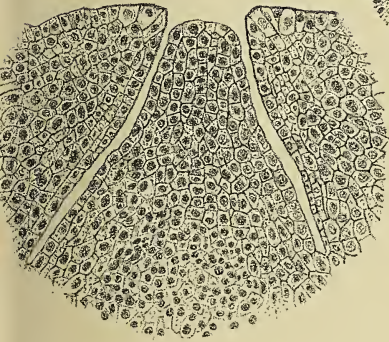
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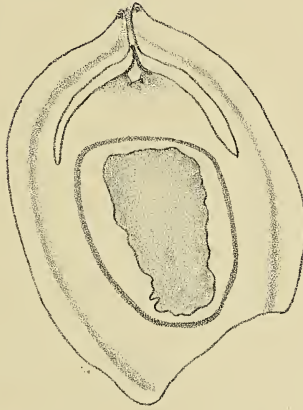
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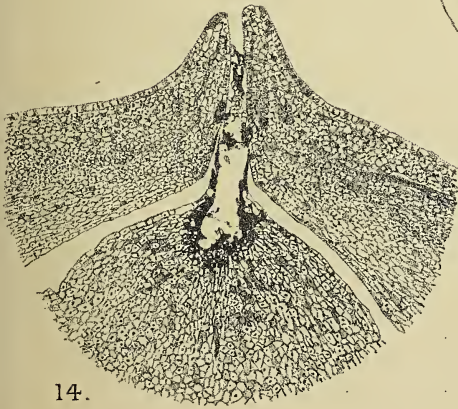
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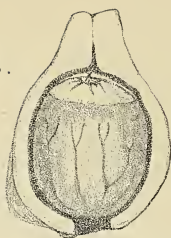




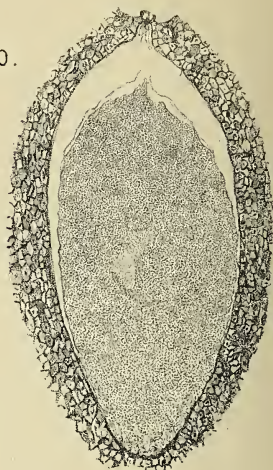
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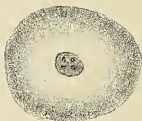
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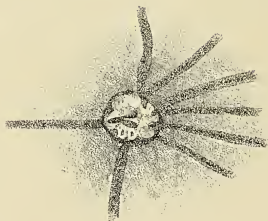
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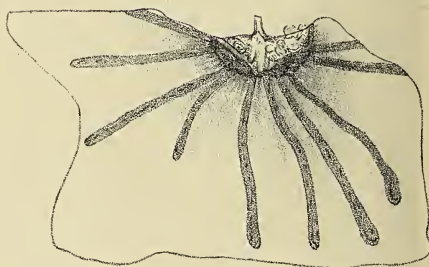
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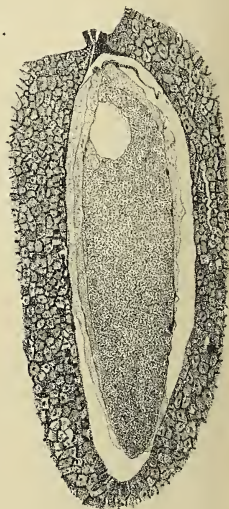
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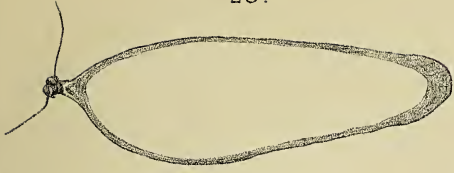


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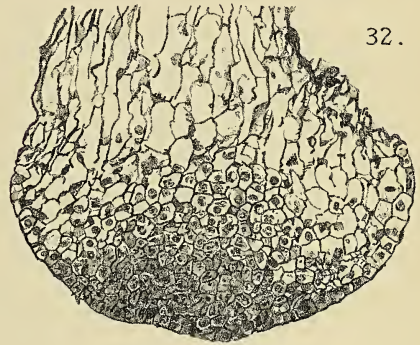
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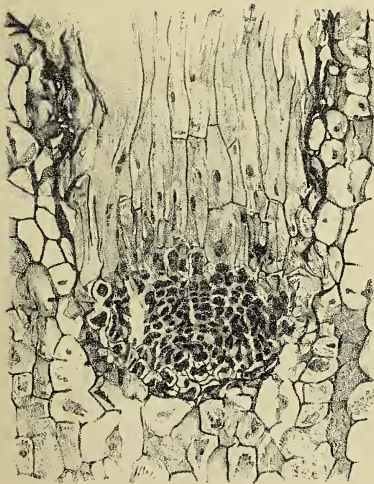
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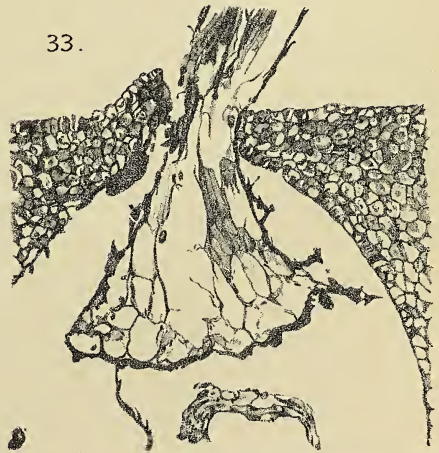
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## NOTES.

**OBSERVATIONS ON THE EFFECT OF DESICCATION OF ALBUMIN UPON ITS COAGULABILITY.** By Prof. J. BRETLAND FARMER, M.A., F.R.S.<sup>1</sup>—It has been known for some time that it is possible, under certain circumstances, to expose seeds to the influence of high temperatures without thereby necessarily destroying their power to germinate. Some experiments in this direction were conducted at the Royal Gardens, Kew, some years ago by Dr. Morris, but the results, although of much interest, do not appear to have been published. However, the seeds were exposed to the action of boiling water, and even to a higher temperature in an oven, without losing their ability to germinate when the ordeal was over.

It has been noticed, in heating seeds in water, that if the seed-coat through any cause becomes ruptured, or if it softens and swells, the seeds which are thus affected are incapable of manifesting any further evidence of vitality. It appears to me that a fair inference to be drawn from these facts is that the admission of water to the living cells is a potent factor in bringing about their death.

Jodin<sup>2</sup> has recently communicated some facts which point to the same conclusion. He exposed seeds of pea and cress to a temperature of 98° C., and found that unless great care had been previously exercised to ensure the dryness of the seeds, they were all killed. When they had been previously dried he succeeded in subsequently germinating 30 per cent. of the peas and 60 per cent. of the cress seeds. Perhaps the disproportion in favour of the latter may, at least in part, be ascribed to their small size, and consequently to the less difficulty in sufficiently drying the seeds.

<sup>1</sup> From the Proceedings of the Royal Society, Vol. lxvi, with a postscript.

<sup>2</sup> Comptes rendus, 1899.

It would seem to follow from what has been said that the instability of the complex molecular structure of which living organisms are made up, may be lessened by appropriate desiccation, but the substances concerned are too complex to render themselves readily accessible to inquiry. It appeared, however, that it might be worth while to study the effects of desiccation on albumin from this point of view. Albumin is not only a highly complex proteid, and perhaps in some respects akin to protoplasm itself, but it is one which gives tolerably definite heat reactions. It is in connexion with the last-mentioned point that the facts detailed in this paper are specially concerned.

It is of course known that albumin in a watery solution is readily coagulated on heating to a certain temperature. This temperature, however, is not necessarily constant for even one type of albumin, doubtless owing to the readiness with which it undergoes change. Thus albumin obtained from different hens' eggs will often be found to coagulate at different temperatures, and the differences appear, in part at any rate, to be connected with the age of the egg. I have found in the case of freshly-laid eggs, that the characteristic opalescence which marks the early stages of coagulation may set in as low as  $60^{\circ}\text{C}.$ , the clotted coagulum being fully formed at  $64^{\circ}\text{C}.$  The heat was applied by means of a large water-bath, so as to ensure its being as uniform as possible. Another sample of albumin from a different egg tried simultaneously and under the same conditions, only exhibited opalescence at  $65.6^{\circ}\text{C}.$ , and coagulated completely at  $68^{\circ}\text{C}.$

The albumin on which most of my experiments were made, was obtained from Merck, of Darmstadt, and was sent as dried egg-albumin. It readily dissolved in water, with the exception of a little flaky insoluble portion, which was filtered off. The solution had a low coagulation-point, the opalescence appearing at  $60^{\circ}\text{C}.$ , and the clot at  $62^{\circ}\text{C}.$  to  $63^{\circ}\text{C}.$ <sup>1</sup> Filtering off the clot and testing the filtrate at higher temperatures yielded no further coagulation.

If a sample of this (dry) albumin be placed in a flask, the mouth of which is furnished with a cork and attached to a set of drying-tubes, and the temperature of the flask raised to  $80^{\circ}\text{C}.$ , a short exposure, of at any rate two to three hours, is enough to completely alter the

<sup>1</sup> It is of course known that several factors affect the coagulation-point. The figures given represent those obtained in my experiments, which were all kept as uniform as possible so as to eliminate the factor of variability.

albumin. The object of the drying-tubes is to prevent any more moisture than is already present in its substance reaching the albumin from the steam or from any other source. Thus heated, the albumin is found to have become insoluble in water, and in fact to have undergone a change corresponding to coagulation.

If, however, the albumin be carefully *dried* before being subjected to these conditions, the results are quite different<sup>1</sup>. For the present purpose it was found to be sufficient to expose a thin layer of albumin in a glass dish to a temperature of 52–55° C. in an incubator. This ensures a very thorough desiccation. The process may be hastened by introducing a vessel of sulphuric acid, though this precaution was not found to be necessary. Thus dried, the albumin loses its shellac or glue-like appearance, and easily crumbles to very small particles.

On comparing the solubility and coagulability of this specially dried material with the ordinary sample, no difference could be detected in any respect.

Numerous experiments were made with this dried material, of which the following may be taken as typical. It may be added that the results throughout were almost surprisingly uniform in the different experiments made.

A sample of the specially dried albumin was introduced in a flask so as to form a thin layer over the bottom. The flask was connected with drying-tubes filled with calcium chloride, and with phosphorus pentoxide. The flask was warmed and cooled rapidly several times, in order to cause the contained air to circulate through the drying-tubes.

The temperature of the flask was then raised in a brine bath to 102° C., and kept at this temperature during the whole of one day (six hours). Next day, and without opening or disturbing the apparatus, the temperature was again raised to 107° C., and finally to 110° C. It was maintained between these limits for seven hours; thus the contents of the flask had been for thirteen hours exposed to a temperature of considerably over 100° C.

On testing the albumin it was found to be soluble in water, and in no way, as far as could be observed, did it differ from the unheated material. On gradually warming the solution side by side with a

<sup>1</sup> Lehmann incidentally observed that albumin dried *in vacuo* could be heated to 100° C. without its power of solution and subsequent coagulation being thereby impaired.



similar solution of the unheated albumin, both became opalescent at a temperature of  $60^{\circ}\text{C}.$ , and both were completely coagulated at  $62^{\circ}\text{C}.$

It thus appears that, if precautions are taken to ensure appropriate desiccation, it is possible to heat albumin for, at any rate, thirteen hours to a temperature varying between  $102$ – $110^{\circ}\text{C}.$  without producing any obvious change in its ultimate molecular (or micellar?) structure. It made no difference to the result whether the heat was gradually or rapidly applied. Thus, in one experiment, the temperature was raised from  $50^{\circ}\text{C}.$  to  $103^{\circ}\text{C}.$  in fifteen minutes, and in other examples the flask was withdrawn from the hot bath, cooled, and suddenly reimmersed. How much higher the temperature could be raised without producing an obvious effect, I am not prepared to say; nor did I investigate the action (if any) which might possibly be produced by a much longer exposure to heat within the limits already mentioned. This formed no part of my object, which was primarily to try to get a point of comparison between the complex seed and the simpler but still very complex proteid.

Other experiments were made in order to test the sensitiveness of the albumin to small quantities of moisture.

For this purpose, two flasks attached to drying-tubes were used, one of them serving for a control experiment, and remaining unopened until the end. The other was opened three times, and a small sample taken out each time. By this means the ordinary air of the room obtained complete access to the albumin. The duration of the experiment was ten hours. The first sample was withdrawn after the flasks had been heated to  $102^{\circ}\text{C}.$  for three hours; it dissolved and coagulated normally. A second sample was withdrawn after three hours more, and it was found that whilst it dissolved and became opalescent on heating to  $60^{\circ}\text{C}.$ , the coagulation change did not at once set in, but the opalescent solution became more milky and of a deeper fog-yellow by transmitted light, finally coagulating at about  $68^{\circ}\text{C}.$  A third sample taken out at the close of the experiment (i.e. four hours after the last opening of the flask) also dissolved, became slightly opalescent at about  $64^{\circ}\text{C}.$ , but did not coagulate even at  $90^{\circ}\text{C}.$ , although the opalescent milkyiness became very pronounced. Viewed by transmitted light, the solution was translucently yellow. Even boiling failed to produce anything which could be fairly termed a coagulum. It appeared probable that the admission of watery vapour

had permitted the inception of the changes which normally, at high temperatures, result in coagulation; but in this case they were arrested, some precursor of alkali-albumin being probably produced, as is often the case on slowly coagulating albumin solutions. Under these circumstances, however, the entire mass of the albumin had undergone this change. This supposition turned out to be correct, for the addition of a trace of acetic acid at once caused the solution to be susceptible to coagulation at about 60–62°C.<sup>1</sup> Hence it is fair to infer that although the slight amount of moisture introduced during the opening of the tube did not suffice to enable complete coagulation to occur, it did permit the early changes to begin, and to slowly, and in a modified way, to affect the entire mass. This experiment was repeated several times, and always with the same result.

It seems difficult, in the light of the foregoing observations, to resist the inference that in the complete absence of moisture, albumin may be reduced to a state of relative molecular (or micellar) immobility; the rearrangements which, in the presence of water and at a sufficiently high temperature, normally take place in its ultimate structure being held in abeyance during the suspension of the essential condition of the presence of sufficient moisture. The substance is brought, so to speak, into a static condition; chemical or physico-chemical change is inhibited, just as is an interaction between phosphorus and oxygen when conditions of complete dryness obtain. It is tempting to extend these considerations to the case of seeds and spores, e.g. of certain Bacteria, and to ask whether similar conclusions may not be fairly assumed to obtain there, for it may well be a fact that the protoplasm, like the albumin which is at any rate akin to it, when sufficiently desiccated withstands conditions which otherwise would certainly promote chemical disintegration. They, too, appear to be reduced to a 'static' condition by drying, and the researches of Romanes<sup>2</sup> indicated no measurable chemical change as proceeding in them under these circumstances; and, again, the investigations of Brown and Escombe<sup>3</sup>, and of Sir W. Thiselton-Dyer<sup>4</sup>, have also rendered it difficult to believe, that, when subjected to the other end of

<sup>1</sup> A solution of albumin treated with a very small quantity of a dilute solution of potash undergoes a similar change. The substance formed is not true alkali-albumen, since no precipitate is produced on neutralizing, whilst a true coagulum appears on heating this neutralized solution.

<sup>2</sup> Proc. Roy. Soc., vol. lvii.

<sup>3</sup> Ibid., vol. lxii.

<sup>4</sup> Ibid., vol., lxx.

the scale of temperature, any metabolism can really be proceeding. In these cases the molecular machinery of life is all present and intact, but the *manifestation of vitality*, as measured by chemical movement and by a change in the condition of energy, is absent. But such a state differs widely from death, seeing that when the conditions favourable to the continuous progress of those reactions which are associated with vitality are restored, the organism proceeds to work in the normal manner once more. Similarly the albumin heated in the desiccated form retains, instead of changing, that particular molecular condition which enables it, on restoring the essential conditions of moisture, to coagulate in a normal fashion when heated to a suitable degree of temperature.

Since the above observations were made, I have been able to experiment with some vegetable albumin, also obtained from Merck.

The substance is in the form of a dry powder, and clearly contains far less water than does the ordinary dried egg-albumin. And in correspondence with this fact it is found to be easily dried in air at a temperature of about 40° C., after which it may be heated in a flask under the same conditions as the egg-albumin, with precisely similar results, allowance being made for the much higher temperature (about 77° C.) at which coagulation occurs. The easy desiccation of the vegetable albumin is perhaps of special interest in considering the power of resistance displayed by seeds when subjected to the action of high temperature.

**ON NUYTSIA FLORIBUNDA, R. Br.**—Originally described by La Billardière (1804) as a *Loranthus*, this plant was subsequently separated from that genus by reason of its peculiar fruit—a dry drupe with the pericarp in three longitudinal wings—by Robert Brown in 1831, who renamed the plant *Nuytsia*, after the Dutch navigator of the Swan River, Peter Nuyts.

*Nuytsia* is especially interesting as a member of the Loranthaceae which is said to have an independent existence, the plant often developing into a tree 10–12 metres in height. Its distribution is extremely limited, being confined to the Swan River region of Western Australia, in which locality the plant is known to the inhabitants as the 'Fire-Tree' on account of its masses of bright orange-coloured flowers.

The seedling exhibits a very unusual feature in having three



cotyledons, a fact apparently first observed by Bidwell<sup>1</sup>. Beyond this peculiarity very little appears to have been known of the plant until quite recently.

In May, 1899, seeds of *Nuytsia* were obtained from Western Australia and were successfully germinated at the Royal Botanic Garden, Edinburgh. The young plants have grown vigorously up to the present, and there appears every reason to hope that they will reach maturity under cultivation.

Fig. 10 represents one of these seedlings about six months old. The most conspicuous feature is the three large fleshy cotyledons, which are persistent for a considerable time, and are much longer and wider than the numerous foliage leaves so far produced. The latter are oval in section, almost round, and taper gradually to a point. While the majority of the seedlings at Edinburgh show three cotyledons, a few possess four; but the fact that in the latter case two of the cotyledons are smaller and appear somewhat malformed, seems to indicate that three is the normal number. The root at present shows no special peculiarities either in external character or in its anatomy.



Fig. 10. *Nuytsia floribunda*, seedling.

Van Tieghem<sup>2</sup> has recently described at some length the anatomy of *Nuytsia*, but there are some abnormalities observable in the seedlings grown here and in material of the matured plant received in spirit from Australia, which appear to have been absent in his specimens, and to these I will direct attention.

An unusual formation of a superficial cambium in isolated patches is a conspicuous feature in the preserved material obtained from Australia. On most of the leaves, as well as here and there on the

<sup>1</sup> Bidwell, Ann. Nat. Hist. viii, 439.

<sup>2</sup> Van Tieghem, Bull. d. l. Soc. Bot. d. France, 1893 and 1898.

stem, and even on the bracts of the persistent involucre, small brown patches occur. They are irregularly distributed, and appear to indicate signs of some disease. Sections taken through a portion of the leaf including one of the brown spots, show the latter to consist of several layers of peripheral cells with no contents, the walls being suberized. Below these, and cutting them off from the rest of the leaf, occurs a well-marked cambium—the whole having somewhat the appearance of a lenticel. These structures may occur on either surface of the leaf or at the margin. It is difficult to surmise the cause of this actively dividing cambium, as there is no trace of fungus; but it might possibly be due to the bite of some insect injuring the epidermis, the subsequent suberization and the cambium below, protecting the inner tissue. It was later observed that many of the seedlings in cultivation here, showed precisely similar formations, with the exception that they were, in many cases, of much larger extent. The first sign of the development of these structures in the leaf of the seedling is a moist glistening appearance on the external surface, as if the epidermis had become porous to moisture. Subsequently, in many cases, mucilage in large drops is secreted and lies on the surface of the leaf in irregular masses over the apparently injured epidermis. In the third or fourth layer below the epidermis a cambium is now formed, which gradually curves up in all directions until it meets the uninjured epidermis around the abnormal portion, completely cutting off the latter from the rest of the tissue. The walls of the outer cells then become suberized and appear a light brown colour. In some cases the cambium is formed in sub-epidermal layers nearly all round the leaf, giving at first sight an impression that it is by this means that the leaf increases in circumference. The formations are perhaps connected with the environmental conditions under which the seedlings are grown, such as an atmosphere too moist, since the method of cultivation of the plant is naturally of a somewhat experimental nature at present.

The non-parasitic nature of *Nuytsia* is still an undecided point. Differing from its parasitic allies in its anatomy and terrestrial habit, it shares with them the same degradation in the structure and development of the gynoeceium. It is obvious that the plant might be terrestrial and attain to its large size and yet be semiparasitic on the roots of other plants. In response to Von Mueller, W. Webb, of King George's Sound, reported that the roots of the mature tree

do not penetrate deeply, and that he never found the roots attached to those of other plants. Should the young plants in cultivation here, and elsewhere, continue to thrive, it may be possible to make experiments and to satisfactorily elucidate the question.

J. H. BURRAGE, Edinburgh.

**ON THE STRUCTURE OF THE STEM IN TWO SPECIES OF LYCOPODIUM.**—Among the few species of *Lycopodium* possessing dimorphic leaves, *L. volubile*, Forst., and *L. scariosum*, Forst., approach very closely to the habit of most species of *Selaginella*. That this is only a general resemblance is seen when one examines the arrangement of the leaves in *L. volubile* in detail. In the stouter branches of this species the leaves are not dimorphic; they are all small and similar, and cannot be referred to a definite phyllotaxy. There may be as many as twenty-two leaves from one to the next in the same vertical line, and they are arranged in an irregular manner, some at considerable intervals, a few of them in pairs, and others crowded into a short spiral, which may be succeeded by a few leaves forming a spiral running in the opposite direction. In the crowding of the leaves there is a tendency towards the formation of pseudo-whorls.

In the smaller branches (roughly those of the three highest orders) the *Selaginella*-type of habit is found; this may be called the distichous region. There are here two series of large leaves extended in one plane, but, judging by the insertion of these leaves, they probably represent four orthostichies, which approximate to two in the upper region. Besides these, there are also about three ventral and three dorsal orthostichies of small leaves with their points directed forwards. This species is thus much more complex than *Selaginella*, which has only two rows of large leaves, and two dorsal rows of small ones. Towards the tips of the branches of *L. volubile*, the smaller leaves become reduced in number. Thus in the lower distichous region there are often three small leaves (two dorsal and one ventral) to each pair of large leaves, while, near the tip of a branch, there may be only two dorsal and one ventral leaf to a length of stem bearing *two* pairs of large leaves. The phyllotaxy of the cone is  $\frac{2}{7}$ . The other dimorphic species of *Lycopodium*<sup>1</sup> have various numbers of

<sup>1</sup> Baker, Handbook of Fern-Allies.



orthostichies, *L. complanatum*, Linn., having four, but all those examined (viz. *L. carolinianum*, Linn., *L. Wightianum*, Wall., *L. complanatum*, Linn., *L. scariosum*, Forst.) appeared to differ from the *Selaginella*-type either in leaf-arrangement or in the number of orthostichies.

The structure of the stem of *L. volubile* was examined to see whether the distichous habit was connected with any modification in the structure of the stele, but this was not found to be the case. The larger stems have a perfectly normal *Lycopodium*-structure, with several xylem-bands, separated from one another by phloem, and bearing peripheral protoxylem-groups. The stele shows reduction in its size and complexity at each successive branching, just as may be seen in a species of *Lycopodium* with radially placed leaves. In branches of four successive orders the protoxylem-groups numbered seventeen, fourteen, eight and seven respectively. Some of the xylem-bands are free; others fused. They are generally straight, and lie in the plane of branching of the stem, which is also the plane of the large leaves in the distichous region. In the latter region there are mostly only two xylem-bands fused at one end, and bearing seven or eight very prominent protoxylem-groups. It may be mentioned here that the mature sporangium in *L. volubile* is axillary in position, and has a well-marked stalk, as in *L. Selago*, Linn.<sup>1</sup>

*L. salakense*, Treub, is not one of the dimorphic species, but has small, sparse, adpressed leaves on its stem, and crowded leaves on its branchlets in eight orthostichies or sometimes in whorls of five at the apices<sup>2</sup>. It is mentioned here because it differs from other species of *Lycopodium* in the structure of its stem. The xylem of the stele does not form well-marked bands, but the tracheides are arranged in small groups and curved uniseriate rows, mostly rounded off, and separated from one another by a network or labyrinth of phloem. The structure has consequently a deceptive resemblance to that of a *Gleichenia*. In the larger branches the protoxylem-groups also are unusual, in that they are very broad tangentially, so that they have the appearance of a nearly continuous peripheral band, which is interrupted at intervals by peripheral groups of phloem. The phloem is thus sometimes completely shut in by xylem for considerable distances. The above structure appears to belong to the type of

<sup>1</sup> Bower, Morphol. of Spore-producing Members, Phil. Trans. 1894, p. 511.

<sup>2</sup> Treub, Ann. Jard. Bot. de Buitenzorg. vii. p. 141.

*L. squarrosus*, Forst., as described by C. E. Jones<sup>1</sup>. The material of *L. volubile* and *L. salakense* examined consisted of herbarium specimens collected by Raciborski in Java.

L. A. BOODLE, Jodrell Laboratory, Kew.

**THE VASCULAR STRUCTURE OF THE OVULE OF CEPHALOTAXUS.**—The seed of *Cephalotaxus* possesses a thick coat, consisting of an outer fleshy and an inner woody layer, thus perfectly resembling that of *Ginkgo* and the Cycads. This outer fleshy layer I regard, with Čelakovský, as homologous with the fleshy aril of *Taxus*. Strasburger has found that the vascular bundles traversing this fleshy portion, one on either side of the seed, are characterized by their *inverted orientation*, i.e., by having their xylem directed outwards, and their phloem inwards towards the woody layer of the seed-coat. This peculiar orientation of the bundles tends to indicate that the outer fleshy layer of the seed-coat is equivalent to a *ligular* structure. When ligules are sufficiently well-developed to possess a vascular strand, the orientation of the latter must necessarily be inverted in relation to the foliar organ on which they are inserted. The ligules of *Isoëtes* and Lycopodiaceae have been regarded by Čelakovský and others as homologous with the *outer integument* of ovules; the velum of *Isoëtes* and Ferns being homologous with the inner integument. This view, which is most probably correct, would lead us to regard the outer fleshy layer of the seed-coat of *Cephalotaxus* as an outer integument, for its bundles are shown to exhibit the orientation required by those of a ligule.

Unfortunately, the aril of *Taxus*, which Čelakovský also regards as an outer integument, possesses no vascular bundles with which to compare those in the ovule of *Cephalotaxus*. But yet another character, which Strasburger does not appear to have remarked, in the outer integument (as I prefer to term it) of *Cephalotaxus* calls for special attention. As above stated, there is a single bundle on each side of the seed. It is greatly extended in the tangential direction, and situated on the innermost side of the outer and in close proximity to the woody inner integument. The secondary centrifugal xylem of the bundle is two or three layers thick. Besides this, however,

<sup>1</sup> C. E. Jones, British Association, Section K, 1898. Ann. of Bot. Vol. xii, p. 558.

(and this is the point of my whole note), *well-developed centripetal xylem* occurs, the tracheides composing it extending along the whole tangential face of the centrifugal xylem in considerable quantity. A very interesting and important point in connexion with this diploxylic structure is the fact that there are two sets of protoxylem, as has been also shown for bundles of a similar structure in the sporophyll of *Cycas*, peduncle of *Stangeria*, &c. One of these protoxylems is attached to the centrifugal, the other to the centripetal xylem. The significance of the fact is this: that it indicates clearly, in my opinion, the derivation of a collateral bundle possessing such a structure from a concentric strand of which the phloem, which in the ancestors of the plant was attached to the outer side of the 'centripetal' xylem, has completely disappeared. This is also my explanation of the occurrence of centripetal xylem elsewhere, as in the peduncle of some Cycads, and the foliar organs of Cycads and Coniferae. I regard *Cephalotaxus* as the most ancient of the Coniferous genera, and therefore, supposing the Coniferae to be derived from some ancient Fern-stock, we should expect to find it exhibiting characters in its most primitive organs similar to those which we find in Cycads and *Ginkgo*. Now the two most primitive foliar organs of any plant are, to my mind, the *cotyledon* and the *ovular integument*. In the former I have already shown the absolutely Cycadean character of the structure of the vascular bundle, with its enormously developed centripetal xylem. In the latter a similar diploxylic structure of the bundle, as has been shown above, also predominates. Two conclusions may be drawn from these facts, viz., (1) That *Cephalotaxus* is the most primitive of the Coniferae; (2) That this genus forms in some measure a connecting link between Cycadaceae and Coniferae, and helps us to trace, however faintly, a fragment of the line of descent of the latter group.

I am much indebted to Prof. F. W. Oliver, of University College, London, for the supply of material for this research.

W. C. WORSDELL, Kew.

**ON THE PRESENCE OF VERMIFORM NUCLEI IN A DICOTYLEDON.**—In consequence of the interesting communication of Nawaschin and Guignard concerning the fusion of a vermiform nucleus with the polar nuclei in *Lilium Martagon*, I undertook last



spring, at the suggestion of Professor Farmer, an examination of the embryo sac of *Caltha palustris*.

The material I obtained from Hackbridge, and pickled on the spot in Flemming's fixative.

After cutting a great many ovaries, which yielded almost all stages before and after fertilization, I at last hit upon the exact fertilization stage, and in an embryo-sac, which shows most perfectly the somewhat attenuated S-shaped male nucleus just touching the ovum, is an excellent instance of a much coiled vermiform nucleus wrapped round the polar nucleus. The pollen-tube is also present. In a neighbouring embryo-sac, the male nucleus is actually fusing with the ovum, while there is a most suggestive polar fusion. Normally the polar nuclei unite before fertilization, and although one might readily suppose that this process could be delayed, it is impossible to imagine that this alone could give the appearance seen. For in addition to an irregular nuclear mass—the polar nuclei—is another nucleus of different character, and without a nucleolus, closely adpressed to it. All the other nuclei of the sac are present.

These, and many other similar cases of polar fusion, were sufficient to convince me that a fertilization of the polar nuclei by a vermiform nucleus actually took place in *Caltha* as in *L. Martagon*; but as fusion had begun in most instances it was often impossible to follow the outline of the vermiform nucleus in its entirety.

However, in another ovule of the same ovary that gave the embryo sacs just described, and only a few sections off in the microtome series, is another perfect instance of a vermiform nucleus, the sharp coils of which can be followed quite distinctly.

I have this spring very carefully pickled a quantity of new material, for the purpose of obtaining a more complete series of sections showing, if possible, the fusion in all stages; and of determining if any points of difference exist in this matter between *Caltha* and *Lilium Martagon*.

The work has been done hitherto at the Royal College of Science, South Kensington, and I wish to thank Professor Farmer for his great kindness and assistance, and also Miss Sargent for her invaluable advice.

ETHEL N. THOMAS, Reigate.

ERRATUM.

Annals of Botany, vol. xiv, p. 123, line 14, for *ovum* read *centre of the ovum*.

# Sexual Reproduction in *Pyronema confluens* and the Morphology of the Ascocarp.

BY

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With Plates XIX—XXI.  
—♦—

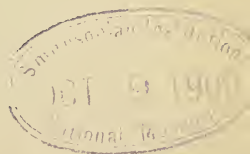
## INTRODUCTION.

THE very interesting researches of Stahl (32) on the sexuality of the Collemaceae have received full confirmation from a number of sources, and the doctrine that a type of fertilization similar to that in the red Algae is to be found very generally among the Lichens must be regarded as well established. The nuclear phenomena involved in the fertilization have in no case as yet been worked out, nor is the account of the cell-fusions complete and consistent, still the existence of trichogyne, archicarp and spermogonium, and their interpretation as functional sexual organs can hardly be doubted, in view of the mass of evidence that has been gradually accumulated.

Krabbe's work on *Cladonia*, *Baeomyces*, and *Sphyridium* indicates that these forms may possibly be without sexual organs. Krabbe (20) investigated *Cladonia* very carefully, and claims that in this genus the ascogenous hyphae are part of the same system of branches from which the paraphyses arise. He notes, that the ascogenous hyphae arise only at one period in the development of a podetium, and that after a certain number have been formed simultaneously, no more new ones arise except by the branching of those already

[Annals of Botany, Vol. XIV. No. LV. September, 1900.]

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present. This is a remarkable condition, assuming that ascogenous hyphae are only modifications of those that form the vegetative thallus. It would be expected that they might bud off at any time from the vegetative hyphae, and in such positions as would be most convenient for the development of asci in the hymenium. Krabbe further describes the ascogenous hyphae in the podetium-stalks as densely staining mycelial branches richly stored with food-materials such as glycogen. He also distinguishes them by the fact that their walls stain blue with iodine, as had been earlier observed by Schwendener.

Still, I am of the opinion that it is a question whether these densely filled hyphae are really the ascogenous hyphae. In *Ascobolus*, *Peziza Stevensoniana*, *Pyronema*, and other forms, the ascogenous hyphae are always empty and frequently collapsed in mature fruits, while the branches destined to produce paraphyses, which are to be developed for the continued growth of the hymenium, agree in their general appearance with Krabbe's description of the ascogenous hyphae. I was myself misled for a time by these late-developing vegetative branches, their prominence and abundant contents leading naturally to the assumption that they were destined to develop asci. As a matter of fact, however, the asci depend very little on food brought by the ascogenous hyphae, which contain little but nuclei, except perhaps at their very tips. If Krabbe's densely staining hyphae are really vegetative, he would very naturally be led to the conclusion that asci and paraphyses are parts of a common system of mycelial branches, since he would readily find paraphyses arising from these densely stained hyphae, and his preconception that they were the ascogenous hyphae would naturally bias his judgement in determining the difficult question as to their actual connexion with the asci.

This possible mistake of Krabbe's is worth such full consideration, since other students are likely to be led into the error of expecting that the ascus-bearing hyphae will be more conspicuous and more densely filled with protoplasm than

those which are purely vegetative. The conditions in *Pyronema*, as will be seen further on, make the true relations of the hyphae in question very clear. A real distinction between ascogenous and vegetative hyphae in all cases so far examined is in the size of the nuclei. Whether this difference will be found in forms yet to be investigated is of course entirely an open question.

As to the actual conditions in *Cladonia* it is to be further noted that Wainio (41) claims to have found its trichogynes. Wainio and Krabbe agree that the podetium is a fruit and homologous with the apothecia of other Lichens so that the carpogonium should be expected to occur at the origin of an entire podetium rather than beneath each area of hymenial surface. It is in the very young podetia that Wainio claims to have found the trichogyne.

Other authors have claimed for various Lichens that the apothecium develops without carpogonium or trichogyne, but the confirmation of Stahl's work on the Collemaceae will certainly necessitate a reinvestigation of all these forms, on the possibility that the sexual organs have been overlooked, and it is not worth while to discuss them further from the standpoint of the existing observations.

Baur's (1) work on *Collema crispum* is of especial interest. He confirms Stahl's (32) observations in all respects. Baur's paper is only a preliminary one but he describes and figures carpogonia and trichogynes of the same type as those of Stahl. He finds that carpogonia, whose trichogynes show no spermatia, grow into vegetative hyphae which may become paraphyses if opportunity is given, thus giving what amounts to an experimental proof of the necessity of fertilization by a spermatium if the asci are to be developed. In four cases empty spermatia were found attached to the end of the trichogyne, whose cells showed in these cases the same degenerative changes described by Stahl. The septa between the upper cells of the trichogyne were swollen, and the lower ones were still clearly broken down. Baur finds that the cells of the carpogonium are uninucleate at first, and obtained

some evidence that later they become connected by openings through their cross walls. Each cell of the carpogonium becomes a source of ascogenous hyphae, so that such pores in their walls are probably to be associated with some part of the fertilization-process. Baur considers that the first cell of the carpogonium is the egg-cell, and that the rest probably function as auxiliary cells, somewhat as has been described by Oltmanns for certain red Algae.

Darbishire (9) confirms Stahl's and Lindau's discovery of trichogynes in *Physcia pulverulenta*. He finds a single nucleus in each cell of the carpogonium and trichogyne, which latter rises about thirty  $\mu$  above the surface of the thallus. The cells of the carpogonium become connected by broad strands of protoplasm so as almost to form a single, many-nucleated cell. Of most interest, perhaps, is the author's criticism of the interpretation of the trichogyne proposed by Lindau (22). According to this author's latest view the trichogyne has no sexual function, but is a boring organ, 'terebrator hypha,' which serves to break a way upward through the thallus for the apothecium.

Darbishire shows that the trichogyne as a rule does not grow upward to the surface of the thallus in the axis of growth of the future apothecium, but rather to one side or the other. In some cases the trichogyne grows parallel to the surface of the thallus, before it turns to follow the most direct line to the surface. Further, its early disappearance and its thin-walled cells with abundant protoplasmic contents are the opposite of what we should expect of a boring organ intended to open a path to the surface of the thallus. The paraphyses grow upward just like other newly formed hyphae of the kind and need no aid in pushing to the surface. I have myself observed and described for *Ascobolus* (16) how the mass of paraphyses acts as a wedge to split the spherical fruit-body at its apex and transform it from the cleistocarpous to the discocarpous type of fruit-body.

Lindau's doctrine seems to be merely a guess, at the best, and when thoroughly tested by comparison with the facts, as



Darbishire has done, it loses all semblance of probability. Lindau (21) in his earlier work has recorded the existence of trichogynes for a considerable series of Lichen-species. His work, taken with Stahl's, Wainio's, and that of the two authors discussed above, makes it certain that organs of this nature are of extremely widespread occurrence among the Lichens. In view of this mass of evidence the negative results of Krabbe, Fünfstück (15) and others, must be regarded as having little weight. It is quite possible that certain genera or families of Lichens may be prevalingly apogamous or parthenogenetic, but that so definite and constant a structure as the ascocarp should be entirely different in its morphological significance in these cases can only be established by positive evidence. It would be most remarkable if such complex structures as the trichogyne, carpogonium, and ascogenous hyphae could be present in *Physcia* and absent in *Cladonia*. At present there is no ground for the view that such apparently closely related forms as are the various genera of the ascus-bearing Lichens must be considered as morphologically unlike in such important parts as constitute the ascocarps, until the last detail of the nuclear phenomena in their reproduction has been worked out. So sceptical an attitude of mind is perhaps stimulative of research, but is not well founded, and its existence at present is simply due to the rivalry of opposing schools.

Similar to Lindau's hypothesis regarding the trichogyne, in the lack of evidence for its support, is Van Tieghem's supposition that the trichogyne constitutes an especial respiratory apparatus for the developing perithecium (37, p. 1166). This hypothesis has received sufficiently trenchant criticism by De Bary (13, p. 237) and need not be further mentioned here.

For the Lichens in general there remains one difficult point. The spermogonia resemble very closely conidial fructifications, which in many Ascomycetes are plainly asexual reproductive organs. It is hence difficult, in cases in which the germination of these spores has not been satisfactorily tested, and the development of the apothecium has not been worked out, to

be sure whether the given structures are male cells or vegetative spores. Brefeld and his pupil Möller (4) have endeavoured to overthrow the evidence for sexuality in the Lichens by laborious culture experiments intended to show that cells which have been claimed to be spermatia in many cases will really germinate, and may, under especial conditions of nutrition, produce rudimentary thalli. Möller describes the germination and growth of the supposed spermatia of species of *Buellia*, *Opegrapha*, *Graphis*, *Arthonia*, and *Calicium*, and concludes that in every case they are merely conidia, and are capable of reproducing the fungal portion of the Lichen by simple germination and growth. He proposes to call the spermatogonia pycnidia and their spores pycnoconidia. Assuming the substantial accuracy of the results as published, though they have not as yet been confirmed by other investigators, it is still quite possible that Möller has pushed too far that conception of sex-cells according to which they must be incapable of further development without conjugation. The inability to develop without conjugation is doubtless an acquired character in the evolution of sexual reproduction from original asexual methods, and it is quite possible in these simple forms, where the spermatia are plainly so similar to known vegetative spores both in their appearance and in the method of their production, that the capacity for independent growth has only been as yet partially lost, so that under conditions of especially rich nutrition, such as Möller's methods furnished, cells which are potential spermatia might return to an original vegetative condition. The weakness of such growth and the failure to achieve it in many cases should have considerable weight, from this standpoint, as evidence of the sexual nature of these spores.

Loeb (23) has shown that such regularly sexual eggs as those of the Echinoderms will still segment regularly and form *Pluteus* larvae without fertilization simply as a result of changing the percentage of a certain salt ( $Mg Cl_2$ ) in the sea water. These eggs are in nature incapable of further development without fertilization, but a relatively simple change in

their environment endows them with capacity for independent growth, or, as Loeb puts it, they contain all the elements necessary for development parthenogenetically, but are hindered by some conditions in their environment. In view of such facts as these it cannot be considered as at all surprising that, under the relatively artificial methods of nutrition employed by Brefeld and Möller, the spermatia experimented with should have been stimulated to the degree of vegetative activity they showed. Indeed in view of Loeb's researches, it would seem impossible to further utilize the test of germination without conjugation as a means of positively determining the sexual or nonsexual nature of reproductive cells. The whole literature relating to the morphology and functions of the Lichen-spermogonia prior to 1884 has been thoroughly reviewed in De Bary's handbook (13, p. 240), and I need not discuss it further here.

In view of all these varied observations, both old and new, the existence of trichogynes and carpogonia in the Lichens, essentially similar to those in the red Algae, must be regarded as established. The most essential phenomena involved in the behaviour of the nuclei are still to be worked out, but the sexual significance of the apparatus from which the ascocarp arises can hardly be questioned by any one not already committed to some other view.

In an attempt to discredit the results of my study of *Sphaerotheca*, Dangeard (8) has investigated the same form and has published a paper with numerous illustrations, quite a number of which might have been copied from my own figures. This paper presents once more the author's theories as to the sexuality of the Ascomycetes, but is also a manifest effort to take account of well-known facts as to the method of development of the ascocarp in the Erysipheae, which he had hitherto seen fit to ignore.

In considering Dangeard's views it is to be noted that there are two problems to be solved in the development of the ascocarp of *Sphaerotheca*. First, does the ascocarp take its origin from a sexual apparatus, consisting of antheridium and



oogonium? and second, are these sexual cells functional, or does the egg develop parthenogenetically? According to De Bary's and my own results both of these questions are to be answered affirmatively. As to the first question Dangeard's figures and descriptions are unmistakable. There is a sexual apparatus formed consisting of oogonium and antheridium as the initial step in the development of the ascocarp of *Sphaerotheca*. As Wager has already pointed out, Dangeard admits this fact, and he repeatedly uses the term antheridium in naming the structure described as such by De Bary. This evidence, coming from an opponent of De Bary's views, must convince the most sceptical that there can be no further doubt as to De Bary's main contention that the ascocarp arises from a sexual apparatus, and is to be interpreted morphologically as homologous in its origin with the sexual reproductive organs of other Fungi and Algae, rather than with their asexual fruit-bodies as maintained by Brefeld, Van Tieghem and others. Dangeard agrees with De Bary that these male and female cells arise from different hyphae, and is inclined to think the hyphae may be from separate mycelia. With the establishment of the existence of an oogonium and egg at the beginning of perithecial development Dangeard's own doctrine that the ascus is an oogonium is left entirely unsupported, unless it is assumed that a sexual apparatus is formed at two stages in the development of the ascocarp, once at its beginning and again when the asci are formed at its maturity. This latter assumption is entirely forced, and without analogy elsewhere among plants or animals. Yet it would seem that Dangeard really supports this view, since he expressly states that the assumption of a fertilization, as he calls it, in the ascus allows perfect liberty of interpretation as to the nature of the archicarps and antheridia, which form the initial cells of the ascocarp, and, as noted above, he continually uses the term antheridium in his description for the cell so named by De Bary. Still it is hard to believe that Dangeard holds seriously to the view that two sets of sexual organs are present in the development

of the ascocarp. Such a view would certainly represent the antithesis of that which has been recently current, according to which the Ascomycetes are entirely without sexuality.

Dangeard professes to find some support for the view that the ascus is an oogonium in the method of reproduction of *Eremascus* and *Dipodascus*. He figures a parthenogenetically produced ascus of *Eremascus* as an illustration of the nature of the ascus in the Mildews and elsewhere. Whether there would be a fusion of nuclei in such a case is of course unknown. The facts as to the parthenogenetic development of some Crustacean-eggs in which the second polar nucleus fuses with the egg-nucleus might afford support for the view that a nuclear fusion is to be expected here, but, granting the relationship of *Eremascus* and *Dipodascus* to the other Ascomycetes, there can be no question that their sexual fructifications correspond to the entire ascogonia and asci of such forms as *Sphaerotheca*, *Erysiphe*, *Pyronema*, *Eurotium*, &c., and not to a single ascus in one of these fruit-bodies. The gamete-cells of *Eremascus* and *Dipodascus* correspond to the initial sexual apparatus of the mildews and *Pyronema*, and not to the pair or pairs of nuclei in the ascus. This is the interpretation given by Eidam and Lagerheim themselves, and Dangeard brings no evidence against it. Indeed, in his whole paper, Dangeard seems to be seeking to save his original proposition, that the ascus is an oogonium, against the evidence of his own observations on *Sphaerotheca*. The whole argument seems like an elaborate attempt to escape from the unenviable position of having proposed a theory as to the sexuality of the Ascomycetes, which simply ignored a mass of fully described, easily verifiable facts in the development of so simple a form as *Sphaerotheca*.

On one point as to the general structure of the ascocarp of *Sphaerotheca*, Dangeard disagrees with me and holds a position nearer to that of De Bary. He finds that the mature ascogonium consists of only three cells, the middle one of which forms the ascus, while I have found regularly five or six cells in the mature ascogonium, of which the penultimate

produces the ascus. This difference, as well as Dangeard's failure to find the conjugation of the antheridial cell and oogonium, is due, I am convinced, to the fundamental weakness of his method of studying the young fruits. I have repeatedly proved in my own experience, as have many others, that it is impossible to get clear views of the fruits, resting as they regularly do on and in an intricate web of vegetative hyphae, except by slicing this mass into thin and transparent microtome-sections.

To the second general question noted above, as to whether the sexual apparatus described is functional, Dangeard replies in the negative. He fails to find any evidence of a conjugating pore between the antheridium and oogonium. In some cases the antheridial cell and nucleus degenerate early while the oogonium is still uninucleate, though in others the antheridial nucleus is still to be found in the antheridium after two nuclei are present in the ascogonium; Dangeard claims to have examined so much material that this purely negative result must be accepted as a final and indisputable proof that the conjugation which he fails to find does not exist. In spite of this certainty, however, I am quite convinced that a more protracted and painstaking search and better methods would have brought to light the stages in development which Dangeard failed to find. Any one who has worked on fertilization-phenomena, either in plants or animals, knows how extremely difficult it is to bring together a complete series of the stages involved, and that negative evidence in such cases has very little weight. De Bary attacked the problem with essentially the same methods of preparation as were used by Dangeard, and failed at exactly the point at which Dangeard has failed, that is in discovering the conjugation-pore between the male and female cells. Dangeard's investigation leaves the problem where De Bary left it.

Wager (40) has pointed out the possibility that Dangeard had a parthenogenetic form of *Sphaerotheca* before him, but I am not inclined to accept this view, and am still convinced that he has failed to find the fertilization-stages because



of his reliance upon antiquated methods of preparation. Dangeard assumes that the nuclear fusions in the ascus are much more easy to find than those in the oogonium. This is true for the larger cup-fungi where scores of asci can be mounted in a single preparation, since the larger number increases proportionally the chances of finding the particular stage in question, but it is not true in *Sphaerotheca*, where each ascocarp produces only a single ascus. I have found it just as easy to discover the conjugation-pore and true sexual fusion of the male and female nuclei in *Sphaerotheca* as to find sections showing the nuclear fusion in the ascus.

Dangeard evidently considers the fact that the male nucleus is smaller than the egg-nucleus prior to the conjugation, as evidence that the former is degenerating. He also regards it as an inconsistency in my figures that the male nucleus at the time of fusion with the egg-nucleus has become as large as the latter. It should not, however, be necessary to point out to a student of fertilization-phenomena that it is an extremely common condition in both plants and animals to find not only the nucleus, but the entire male cell at certain stages much reduced in volume, and that it is not at all uncommon to find that the sperm-nucleus has increased in volume at the time when it unites with the egg-nucleus.

Miss Nichols (25) has also investigated the development of the ascocarps in a number of Pyrenomycetes. If one judges by the impression produced on her reviewers, Miss Nichols's results, so far as the question of sexuality in the forms she investigated is concerned, must be regarded as somewhat ambiguous. Sydow considers that Miss Nichols's observations revealed not a trace of sexuality in the forms investigated (!), while Wager (40, p. 584) states that her work distinctly supports the view that sexuality is present.

Miss Nichols's account of the development of the ascocarp of *Teichospora* and *Teichosporella* by the division of a single hyphal cell to form an oval parenchymatous mass is certainly very interesting. As I understand her description

it agrees very exactly with the method of development described by Bauke (2) for the ascocarp and pycnidium of *Pleospora*, though Miss Nichols does not refer to this author. De Bary had already expressed the view regarding this method of ascocarp-formation, that it is quite fundamentally different from that in other Ascomycetes and is to be interpreted as a case of apogamy. We shall need to know more than Miss Nichols describes of the nuclear phenomena in these initial cells, and of the development of the ascogenous cells before an interpretation of this method of ascocarp-formation can be attempted. The account given of the sexual organs in *Ceratostoma* and *Hypocopra* is not widely different from that of Woronin for *Sordaria*, as Miss Nichols points out.

Because of the large size and conspicuous occurrence of the sexual apparatus above the substratum, *Pyronema* was one of the first Ascomycetes to be thoroughly investigated. R. and C. Tulasne (35) observed in 1860 the presence of large globular vesicles on the mycelium, which they recognized as the first indication of approaching fruit-formation. They called these vesicles macrocysts, but failed to discover their sexual character or that they were of two kinds.

De Bary (11, p. 11) in 1863 published a further account of the Fungus in which he points out that these vesicular cells are in pairs consisting of a thicker flask-shaped oogonium and a club-shaped antheridium. He describes the groups or rosettes consisting of several of these paired bodies as arising from one or a small number of mycelial twigs. He also observed a gradual disappearance of the protoplasm from the pairs as they become enclosed in the enveloping hyphae of the hypothecium. He also finally settles the point that there is no fertilization of the asci by the paraphyses, and throws doubt on the existence of the conidial fructification earlier described by Tulasne. He was unable, however, to find an actual fusion between the peculiar paired bodies which he describes.

In this respect he left the matter exactly in the condition

in which he left our knowledge of *Sphaerotheca*, having in each case described the sexual apparatus without being able to prove a fusion of the sexual cells. At that time, however, it was supposed that mere contact of the male and female cells might be sufficient for fertilization. De Bary was naturally unable to determine the exact function of the intermediate cell, or trichogyne, as he later called it. He describes the paired bodies as in intimate contact throughout their whole length, a statement which is corrected by Tulasne (36, p. 218).

In 1866 (36) the Tulasne brothers published further results of their investigations on *Pyronema*, in which they describe the fusion of the paracyst (antheridium) with the tube at the apex of the macrocyst (oogonium). They were able to observe the actual union of the protoplasm of the connecting tube and paracyst-cells through a rounded pore formed at their point of contact. This pore they described as having a thickened border (*bourrelet*). The connecting tube is cut off from the oogonium before its fusion with the paracyst. They also recognized the difficulty of determining which of the two cells is the active organ in effecting the conjugation. In some cases the connecting tube seemed to extend over more than its proportion of the distance necessary to bring the two together. Again the paracyst seemed to anticipate this action of the connecting tube. They further observed, what is quite true from a surface view of the facts, that if the protoplasmic contents of the conjugating cells do influence each other reciprocally, this influence does not produce any appreciable change in their appearance. The interpretation of the fusion they leave uncertain, but point out that the conjugated cells ultimately wither and become empty (especially the macrocysts), while the filaments grow up about them which are to form the ascocarps (*thèques*) of the Fungus. They observed no development of branches from the macrocyst, and apparently supposed that the asci arose from the filaments that build the envelope around it. They give a long series of beautiful figures of the conjugating



groups in all stages of their development, which leave nothing to be desired as to accuracy and detail so far as the surface views of the structures are concerned. In the *Carpologia* the Tulasnes described the formation of asexual spores of *Pyronema*. In the paper under consideration, they correct this error and assign the earlier described conidial spores to *Peziza melanoloma* which they found frequently associated with *Pyronema*.

Kihlmann in 1883 (19) was the first to discover the development of ascogenous hyphae from the archicarp (macrocyst) of *Pyronema*, and thus bring the sexual process here into line with what Janczewski (1871) had already described for *Ascobolus*. Kihlmann made a very careful study of the form, and the account in De Bary's handbook (13) is based on his work. He concludes, although without very positive evidence, that the macrocysts and paracysts of a group arise from separate hyphae. He never observed a macrocyst and paracyst arising from a single hypha. He describes them as arising simultaneously in from six to sixteen pairs in each group or rosette. The paracysts are a few  $\mu$  longer than the macrocysts and about one half as thick. Sometimes two conjugating tubes connect with one paracyst. One case was observed in which the paracyst was branched, though remaining one-celled, and each branch was connected with a macrocyst by conjugating tubes. The conjugating pore between the paracyst and conjugating tube is 3–4  $\mu$  in diameter, and is bounded by strong sutures. He was able, by pressure on the paracyst, to cause a flow of protoplasm through this pore from the paracyst to the conjugating tube. When the pressure is relieved a return flow is set up.

Kihlmann also finds that the cross-wall at the base of the conjugating tube is always formed before the latter fuses with the paracyst, so that a direct mixing of the protoplasmic contents of the paracyst with that of the macrocyst is impossible. He thinks, however, that a diffusion of dissolved substances may take place through this basal wall of the conjugating tube, and that thus fertilization may be effected.

In this connexion he makes the interesting observation that a button-like granule is present on the middle of this basal wall of the conjugating tube in its younger stages on the side toward the macrocyst, and that this granule disappears in older stages. He notes also that it stains yellow with iodine and swells from two to three times its original volume when treated with caustic potash solution.

After the formation of the conjugation-tube the macrocyst swells to two or three times its original volume. Later, branches arise from its surface as papillae, and grow out, piercing between the vegetative enveloping hyphae which are already present. These branches at their point of origin are much thicker than the paraphyses. They are very crooked and soon become septate.

Kihlmann believed that the asci were developed from the ultimate ramifications of these branches, but he was not able to trace the connexion directly from macrocyst to ascus. As these ascogenous branches develop, the macrocyst becomes emptied and vesicular and is not recognizable in the old fruit. At the time when the ascogenous hyphae begin their development, the antheridium and conjugation-tube are still full of protoplasm, which is sometimes very vacuolar. Kihlmann suggests the possibility that the basal wall of the conjugation-tube may become swollen, as do the cross-walls of the trichogyne in *Collema*, and that the disappearance of the granule mentioned above may be associated with this change.

As to the homologies of the parts concerned, Kihlmann agrees with De Bary that the macrocyst is an ascogonium, the paracyst an antheridium, and the branches of the macrocyst are ascogenous hyphae. The whole structure is similar to that in *Collema* as described by Stahl, with the difference that in *Pyronema* the trichogyne is reduced to a single cell, and the ascogonium is also unicellular. The development of several ascogonia to one ascus-fruit is also found in *Physma* and *Collema*. He also considers that the development of the male element as an antheridial branch in *Pyronema*, and as

a spermogonium in *Collema* is due to the development of dioecism in the latter.

Kihlmann concludes, however, that the actual fertilization of *Pyronema* is still an hypothesis. The homology of the reproductive organs is unquestioned, but the possibility that they are not functional still remains, since parthenogenesis and apogamy are widely distributed phenomena. The nature of the sexual process was much better understood when Kihlmann published his work in 1885, than it was when Tulasne and De Bary did their work. Still in spite of the progress that had been made, Kihlmann remarks that he considers it a question whether nuclei are present in the sexual cells of *Pyronema*, and further, whether their behaviour, if present, would show phenomena of decisive worth for settling the question of fertilization.

#### OBSERVATIONS ON PYRONEMA.

The sexual reproductive apparatus in *Pyronema* is the largest and most conspicuous which has yet been discovered among the Ascomycetes. In it we have a case analogous to that of *Sporodinia* among the Zygomycetes, in which the zygosporcs, contrary to the habit of the group, are produced on an aerial mycelium instead of beneath the surface of the substratum. The young ascus-fruits of *Pyronema* are visible with a good magnifier, and their distribution and approximate stage of development can thus be determined with considerable certainty.

I found the form used, which seems to be *P. confluens*, abundant in woods on burned places, especially in half-charred masses of leaves, though it also occurs among damp well-decayed leaves where there has been no fire. When conditions are favourable it is extremely common, and can be obtained in unlimited quantities in all stages of growth. The development of the mature fruits from the time of the appearance of the sexual apparatus only requires a few days. The development of the sexual apparatus itself up to the stage when fertilization occurs requires not more than twenty-four



hours. The fruits are densely packed in small tufts, and are a delicate pinkish or salmon colour when growing among leaves. The mycelium and sexual organs are glistening white, and resemble a delicate frost-like tracery on the blackish substratum. The best material is always found under and among the leaves, so as to be at least partially protected from the brightest light in the cases I have observed, though it is described as growing directly on the ground on burned places in Europe. My material was collected in May and June of 1898 at Lake Forest, and I was able to obtain unlimited quantities of it at that time, sufficient for experimenting with all varieties of fixing-fluids, &c.

As just noted, the material for my investigations was found growing on charred or decayed leaves or leaf mould. Bits of this substratum a few mm. in diameter and richly covered with fruiting bodies, young or old as might be desired, were fixed *in toto*, embedded in paraffin and sectioned for the most part in a plane vertical to the surface of the substratum as it lay when collected. In this way the sexual apparatus was fixed without distortion, as would hardly have been possible had the attempt been made to handle the rosettes of oogonia and antheridia separately. The growth of the mycelium is peripheral, from a centre outward, and the bits of the substratum usually contained fruits of varying ages, the youngest lying near the border of a mycelial growth with progressively older stages toward the centre. The development is, however, so rapid that it is not possible to find the fully mature as well as the youngest stages on any single mycelium. The oldest fruits found on mycelia, at whose edges the rosettes were first appearing, showed only a few partly ripened asci, and conversely those mycelia which showed fully mature pink apothecia at their centres rarely bore fruits as young as the fertilization-stages at their margins. By noting such points as this one is enabled to be sure of obtaining an abundance of material at all stages of development. The substratum on which I have found the *Pyronema* most abundant, makes it especially favourable for sectioning,

the leaves or leaf mould offering little or no resistance to the microtome-knife. More trouble is sometimes occasioned by diatoms, which were found extremely abundant in the substratum in some cases, and indicate the degree of moisture favoured by the Fungus. Blue-green Algae and some Conferva-like filamentous species were also present in many cases. *Pyronema*, as I have found it, is extremely susceptible to drought, a short exposure in the laboratory causing it to wilt and die. These points as to its occurrence are of interest in comparison with Kihlmann's experience. He seems to have found the Fungus abundant in clefts on stones and to have secured successive crops of young fruits by wetting the stones every three to four days.

For fixing my material I used Flemming's solutions, both the stronger and weaker, and also of a strength about half that of the weaker formula. Hermann's and Merkel's solutions were also tried. Of the sublimate acetic solutions both Kaiser's and Wilson's were tried. Merkel's solution was found far superior to all the others as a fixing-agent for the younger stages in the development of the fruits, up to the time of ascus-formation. Solutions containing osmic acid such as Flemming's solutions in any strength seem to reduce the protoplasm, of the oogonium especially, to a dense undifferentiated mass in which even the differential staining of the nuclei was a matter of great difficulty. This action of the solutions containing osmic acid seems due to the presence in the protoplasm of the reproductive cells of certain reserve food-materials whose nature I have not attempted as yet to more fully determine. As a result of their presence young material fixed in Flemming's solution was practically useless. Merkel's solution, however, gave preparations of a clearness which left little to be desired. The sublimate solutions gave well-fixed and unshrunk sections which, however, seemed not well suited to differential staining with the anilin dyes. With iron-haematoxylin this material gave very good figures, but the plain black or blue and white is far less useful than the series of varied shades which can be obtained with such

combinations as the safranin-gentian violet-orange, or acid fuchsin and iodine green, when used on material fixed in Merkel's solution. The earliest stages figured are from preparations stained with acid fuchsin and iodine green. The fertilization-stages were mostly drawn from preparations treated with Flemming's triple stain. For the nuclear phenomena in the ascus the Flemming fixing-solution, weaker formula, and the triple stain were used.

I have mentioned the slight differences in the habit of the Fungus studied from what is described for *Pyronema confluens* in Europe. I have no doubt, however, that our plant is the same species. The general specific characters agree very well, and the reproductive organs in the fresh preparations I have studied might have served for Tulasne's drawings.

The mycelium is made up of cells of varying length which are regularly multinucleate, containing from six to twelve or more nuclei (Pl. XIX, Fig. 2). They are filled rather evenly with protoplasm of a loose spongy structure, there being as a rule no conspicuous large central vacuole. As a whole the mycelium is extremely sparse and loose, never making densely felted layers in my experience. The sexual organs are, however, very abundant, especially at the centre of the mycelium, so that when the ascus-fruits are mature they are frequently densely packed together, forming continuous irregularly circular turfs. Toward the margin of the mycelium the reproductive organs are progressively more sparse, and the resulting fruits stand isolated. The whole arrangement indicates peripheral growth of a mycelium from a central point about which most of the fruits are developed. Such a condition would naturally result if each such radially growing mycelium were the product of the growth of a single spore. Whether this is the true interpretation in every case is doubtful. It is quite possible that this arrangement of the mycelia indicates the relative distribution of moisture, food material, &c., favourable for the life of the Fungus. The centre of most vigorous growth would then indicate a point at which conditions were especially favourable. A filament of a neighbouring mycelium



reaching such a point would, by its vigorous branching, produce a centre of growth and result in a radially arranged mycelium such as I have described. The Fungus certainly spreads by means of loose, cobwebby hyphae over considerably greater areas than are ultimately covered by the apothecia. These scattered hyphae are hardly to be described as definite stolons such as are found in *Rhizopus*, and yet their general function is much the same.

I have never found any traces of asexual reproduction by conidia or otherwise. Sexual reproduction is so prolific apparently, and the ascus-fruits are produced in so short a time, that asexual reproduction has been dispensed with. I have not tried germinating the spores in nutrient media, and it is quite possible if they could be made to grow in this way that some type of vegetation fragmentation might appear, but it is quite certain that such reproduction does not occur, or at least does not play any prominent rôle in the life history of the Fungus in nature. *Pyronema* has developed the opposite habit to that found in *Penicillium*, for example, where the asexual conidia are the predominant reproductive bodies, ascocarps being formed rarely or perhaps never in nature. What has led to such different lines of development in the two forms is as yet an unsolved problem. Doubtless the explanation of the predominance of the *Penicillium* and the comparative rarity of *Pyronema* is involved to some degree at least in this difference in their reproductive habit.

Vertical median sections through a mycelial growth such as I have described show ascocarps in various stages of development, the older near the centre, the younger toward the periphery. The first indication of sexual reproduction, as it has so many times been described, consists in the formation of thickened hyphal branches which tend to stand vertical to the substratum. Thin microtome sections are not favourable for the investigation of the question as to whether these knots of hyphae arise from one or more than one parent branch. The results of a study of the question on living material by the different authors have been already referred

to. The point is not a vital one in settling the question of the existence of sexuality in the form, since differentiation of sex has been shown everywhere in plants and animals to occur at earlier or later stages in the life history of the individual as a result of the influence of factors which are as yet little understood.

A section through these knots of hyphae shows that they are composed of multinucleated cells, with denser contents, but not otherwise different from ordinary vegetative mycelial cells. There is no one-nucleated stage in any of these cells. They are formed by growth and division of multinucleated hyphal cells in general as the cells of *Cladophora* are. The apical cells of these short branches soon show more rapid growth than those immediately below them, and become swollen. They are filled with spherical or oval vacuoles at this stage so that their contents have a foamy appearance as if growth had gone on so rapidly that sufficient protoplasm to fill the swelling cell could not be provided at once. At a very early stage it is possible to differentiate two types of these apical cells, one more spherical—the young macrocyst of Tulasne, which is the oogonium—the other more oblong or club-shaped—Tulasne's paracyst, which is the antheridium.

Very soon a slight elevation or papilla appears on the apex of the oogonium, and this is the beginning of the young conjugating tube or trichogyne (Fig. 3). Both antheridium and oogonium are multinucleated from the start, and the number of nuclei increases by division as the cells grow in size. The nuclear multiplication, however, is out of proportion to the vegetative growth, so that when the sexual cells are mature they contain relatively to their size many more nuclei than do the ordinary vegetative mycelial cells. A broad stalk-cell is usually cut off from the base of the oogonium relatively late in its development, after it has attained its full size or nearly that (Fig. 5). A number of stalk-cells can also as a rule be distinguished at the base of the antheridium (Figs. 6 and 19). The papilla-like beginning of the conjugating tube on the oogonium rapidly elongates with the growth of

the latter (Fig. 4). The club-shaped antheridium is slightly curved over the oogonium, and the conjugating tube grows upward on the broad rounded end of the former. The apex of the antheridium reaches to or a little past and to one side of a line through the long axis of the oogonium and its stalk (Figs. 1 and 14). The conjugating tube lies in this same axis at the start, but coming in contact with the antheridium it applies itself closely to its surface and thus becomes curved and sometimes even hook-shaped to conform to the surface of the antheridium.

It may grow directly up over the apex of the antheridial cell and then bend slightly to the right or left at its tip. More commonly the antheridium extending beyond the vertex of the oogonium, the conjugating tube grows up on one or the other of its flanks, and then bends forward and applies its tip to the former a little above its vertex. Frequently the end of the antheridium bends towards the conjugating tube as if to encircle it so that the tube comes to lie partly enclosed by the antheridium. Sections in such cases make it appear as if the tube cut through the antheridium (Pl. XX, Fig. 14). Antheridium and tube are growing at the same time, and their attraction for each other is apparently mutual. Frequently, the antheridium presses against the tube so that a groove is formed in its side in which the tube lies. The upper margin of this groove may then be developed into a swollen lip. All of these conditions and others have been beautifully figured by the brothers Tulasne (36), and I have not thought it necessary to reproduce them further than I have done in Fig. 1 which is a surface view of a group mounted in glycerine.

The contents of the conjugating tube are at first in direct connexion with the oogonium through its base (Fig. 4). It is multinucleated at this stage and its nuclei are not different from those in the oogonium. Early, however, and long before its tip becomes fused with the antheridium, a cross-wall is formed at the base of the tube, cutting it off from the oogonium. This wall is formed before the sexual cells or



the tube have reached their mature size. During subsequent growth it is interesting to note that the nuclei of the tube do not increase in size as do those in the antheridium and oogonium, so that the diameter of these nuclei of the tube remains regularly less than that of the nuclei of the sexual cells themselves. This condition continues until the nuclei of the tube are about to disintegrate, as will be described below, when they swell up without increase of their contents until they may equal in size the nuclei of the sex-cells, but are very transparent. Whether nuclear divisions occur in the tube after it is cut off I am not certain.

Kihlmann has described a button-like granule on the basal wall of the conjugating tube next the oogonium. I have found in some cases a similar granule or pair of granules (Figs. 12 and 18). They are doubtless connected with some system of pores by which materials pass from one cell to the other. I have found in *Ascobolus* and other *Pezizaceae*, as well as *Pyronema*, a row of granules on each side of the septa of vegetative hyphae in the hypothecium. I have observed them also in the mycelial cells of *Pyronema*, and very strongly developed in the swollen storage-cells of the hypothecium (Figs. 2 and 24).

The tip of the conjugating tube is blunt and rounded at first (Fig. 4), but when mature is always narrowed into a beak or snout-like projection at its apex. This beak is about one half the diameter of the body of the tube and serves as the special organ for fusion with the antheridium. When conjugation is complete the tube at the point where it narrows into this snout is always pressed firmly against the antheridium. The snout on the other hand at first bends slightly up and away from the surface of the antheridium, and then turns sharply down at its tip, which thus strikes the wall of the antheridium again almost at a right angle (Figs. 6, 12, 17, and 21). The tip of the tube is pressed closely against the antheridium wall and the conjugation-pore is then formed as will be described below. This bend in the beak-like end of the conjugation-tube is always well shown when the section

lies in the long axis of the beak, and it gives very much the appearance of the piercing of the antheridial wall by the end of the beak, but taking the appearance and arrangements of the parts as a whole it is easy to see how Tulasne was led to the conclusion, that it is impossible to say whether the antheridium or the conjugation-tube is the more active in accomplishing the fusion. This mutual approach of the two cells is perhaps most conspicuous in surface views of the sexual organs such as Tulasne studied (see Fig. 1). This mutual attraction and bending of both cells might be expected between equal gametes. In the bulk of Fungi which show antheridial branches it is the latter which are the active structures, and in their growth apply themselves closely to the surface of the oogonium, but here the female cell has also developed an organ—the conjugating tube—which is at least equally active with the antheridium in effecting the union.

The mature sexual apparatus shows nothing further than has been already mentioned in the development of its parts. Its structure may be summarized as follows:

The oogonium is a spherical or flask-shaped cell filled with dense protoplasm and many nuclei which are very much larger than those of the ordinary vegetative cells. Its stalk consists of two or three broad disk-shaped cells of which the basal one is a part of the mass of thickened swollen cells forming the base of the rosette. The apex of the oogonium is continued into the narrow conjugating tube which curves upward to unite with the end of the antheridium. The antheridium is a curved club-shaped cell thickest near its upper end and tapering gradually to its base where it is continued into a stalk of one or more cells. The basal wall of the antheridium is as a rule somewhat higher up than that of the oogonium. It follows a somewhat oblique path upward, conforming rather closely to the surface of the oogonium, and its apex is even with, or reaches somewhat past, that of the latter. The mutual relation of these sexual cells in a typical case will be best understood from Fig. 1.

The nuclei of the antheridium are of about the same size as those of the oogonium. The protoplasm of the antheridium stains much less densely than that of the oogonium, owing perhaps to the absence of accumulated reserve materials. As a result the nuclei are more sharply differentiated in the antheridium, since they stain quite as intensely as those in the oogonium. These sexual nuclei are relatively large and very clearly defined in their structure. They contain a very small spherical nucleolus which appears bright red when the triple stain is used. The chromatin forms a conspicuous net of granules and fibres staining blue or violet with the same triple stain. For those who are still sceptical as to nuclear structures in the Fungi it should be said that these sexual nuclei of *Pyronema* are quite as conspicuous and quite as available for study by their staining qualities as are the nuclei of many of the higher plants. Even prior to the fusion of the antheridium and conjugating tube, short branchlets are to be found budding out from the stalk-cells of the oogonium and the neighbouring vegetative cells, and these later grow upwards to form the hypothecium and paraphyses. Whether such branches arise also from the stalk cells of the antheridium is uncertain. They certainly are not present at this early stage, and later after the fertilization has been accomplished it is difficult to make out with certainty the origin of all the hyphae which are combined to form the young fruit-body.

If we turn to the process of conjugation and fertilization in the sexual apparatus just described, it is apparent that to permit the union of the male and female cells in addition to the fusion already mentioned as occurring between the tips of the antheridium and conjugating tube, the breaking down of a second wall at the base of the conjugating tube between it and the oogonium is necessary. The first of these two fusions was discovered by the Tulasne brothers (36) and studied fully by them and by Kihlmann (19). These investigators, however, were unable to observe the breaking down of the basal wall of the conjugating tube, and their



demonstration of a true fertilization, involving the fusion of male and female cells, was hence incomplete. Kihlmann's statement, that true fertilization in *Pyronema* was still only an hypothesis, was in a sense justified from the facts as he was able to work them out. For De Bary who, at the time he worked on *Pyronema*, believed in the possible transmission of a fertilizing influence through a cell wall and without fusion, the case was easier. As a matter of fact, as is shown in Figs. 15 and 15*a*, the second fusion between the base of the conjugating tube and the oogonium does occur, and thus, as will be seen from the following description, all doubt as to the existence of sexuality in *Pyronema* disappears.

If we examine now in detail the changes in the sexual apparatus after it has reached maturity; which lead up to fertilization, we shall find first of all, that an area of protoplasm in the antheridium, in the region where the tip of the conjugating tube is pressed against its wall, is differentiated as a very finely granular disk, and that the nuclei have withdrawn from this region (Figs. 7-12). This finely granular area, although located in the antheridium, is quite similar in shape and consistency to the so-called mouth-piece or receptive spot seen on the oospheres of *Oedogonium*, *Vaucheria*, and other Algae. It is an irregularly lens-shaped mass of hyaline protoplasm aggregated at the point where the first fusion-pore is to be formed. If we examine the beak-like prolongation of the conjugating tube we find that it also contains no nuclei, and its protoplasm is also denser and more finely granular than that further back in the tube (Figs. 6 and 9-12). The differentiation of the protoplasm here is not so conspicuous but is quite as characteristic as in the area opposite it in the antheridium, and it doubtless indicates the differentiation of a special receptive spot equivalent to the hyaline mouthpiece in the Algae referred to above. It is less conspicuous only because it is enclosed in the narrowed beak so that it cannot take on the characteristic lens shape. These areas are evidently especially differentiated as a preparation for the process of fusion and may be regarded as a real

equivalent of the receptive spot in the oospheres mentioned. That such an area occurs in the male cell as well as in the female cell is a further indication, in addition to the facts noted above, that the antheridium in this case is not alone the active element, a mutual reaction being rather induced between both antheridium and conjugating tube. I have not found this receptive spot in the antheridium until after the conjugating tube was in contact with it at its apex. The corresponding area in the conjugating tube is developed as the beak is formed. In their late appearance these structures differ from the receptive spot in the oospheres mentioned, which by some authors have been regarded as centres for the diffusion of substances which serve as a chemotactic stimulus to the male cell to attract it to the egg.

The walls of the antheridium and conjugation-tube, at the point where the beak of the conjugation-tube is closely pressed against the antheridium, break down, and a pore is formed leading from the tube to the antheridium. The process of solution of the wall seems to be a rather gradual one and to consist in the softening and dissolving of the wall-material which swells and seems to spread gradually out into the protoplasm of the beak. Sections at this stage show the hitherto sharply defined walls replaced by a spongy mass, which stains deeply with safranin and seems to be diffusing upward into the beak as fine fibres or ragged shreds (Figs. 8, 9); on the side next the antheridium there is no indication of such diffusion of the swollen wall-material into the fine granular plasm of the male receptive spot. This perhaps indicates that the solvent action on the wall is mainly exerted from the interior of the conjugation-tube.

Ultimately a circular disk of the walls is entirely dissolved away, leaving the roundish pore through which the protoplasm of the two cells becomes perfectly continuous. Irregular deeply staining bits of material remain for some time in the neighbourhood of the pore representing the swollen but not completely dissolved fragments of the broken-down wall (Fig. 10). Tulasne and Kihlmann have both noted the

thickening of the walls around this pore. This thickening is quite conspicuous in sections and seems to be for the most part on the wall of the tube rather than on that of the antheridium. Figs. 9-13 show it extending back over a portion of the underwall of the beak. As a result of this thickening an extremely strong suture is formed between the antheridium and conjugating tube, so that they can be bent and turned up on each other as Kihlmann's figures show without being pulled apart. Without doubt this extra reinforcement of the walls at this point is necessary to withstand the strain which comes with the passage of the nuclei from the antheridium into the conjugating tube. The flow of nuclei from the relatively wide cavity of the antheridium through the narrow pore and beak of the conjugating tube must produce considerable strain on the walls of the latter, and the thick irregular ring-shaped thickening provides against the possibility of a rupture. While the pore has been forming, the centre of the lens-shaped receptive spot of the antheridium has become spongy and vacuolar (Figs. 11-12), and its especially fine granular structure gradually disappears.

During the process of fusion just described the contents of the conjugation-tube have been undergoing a striking change. The protoplasm of the tube has finer meshes and stains less deeply than that of the oogonium. At its first maturity, before the pore is formed, its nuclei are much smaller than those in the oogonium. These nuclei now undergo degenerative changes of quite a characteristic type. Their chromatin content seems to be reduced and is distributed for the most part on the nuclear membrane. For a time the minute red-stained nucleoli become more conspicuous. The nuclei then swell and become extremely transparent (Fig. 6). They may also tend to flow together in groups in the axis of the tube. Finally they collapse or break down into dense strands or shreds which are frequently connected so as to form a coarse, much broken network in the protoplasm (Figs. 8-13). This network stains strongly with safranin and seems quite evenly distributed through the tube with the exception of the beak.



The individuality of the nuclei is entirely lost in this disintegration, and it is quite impossible to determine at this stage just how many or how large shreds have come from a single nucleus. The partial union of the shreds into a network seems to have resulted from the aggregation of the nuclei in groups just before they lose their spherical shape and disintegrate. Having broken down they are apparently once more rather evenly distributed in the cytoplasm, but the fibrous shreds into which they have disintegrated remain more or less connected. This disintegration of the tube-nuclei may be complete at the time the pore is formed or it may be delayed somewhat, so that cases are found where the pore has been formed while the tube-nuclei are still intact (Fig. 7).

When the nuclei of the tube are completely disorganized, a migration of the nuclei from the antheridium through the pore begins. Figure 13 shows a case in which three of the antheridial nuclei have entered the beak of the tube, and the tube-nuclei are broken down into the irregular network described. With the inflow of these antheridial nuclei the entire protoplasmic contents of the tube become still further disorganized. This may go so far at once that the fragments of the tube-nuclei disappear entirely, apparently being assimilated by the inflowing antheridial nuclei and cytoplasm (Fig. 14); or the protoplasm of the tube may become aggregated into a densely staining mass embedded in which the remnants of the nuclei appear as very densely stained lumps and granules. This mass may persist after the entire process of fertilization is complete (Figs. 15 and 17 *a*) in some cases, and it may remain in the tube or it may be carried through into the oogonium after the basal wall of the tube has been broken down. In very many cases it can still be recognized in the tube after the fruit-body is practically mature, and the oogonium and antheridium appear as mere empty shells. The antheridial nuclei continue to pass through the pore into the tube until the latter is quite densely filled (Fig. 14) and sometimes a trifle swollen.

Meanwhile conspicuous changes have been taking place in the oogonium. When mature the oogonium was filled with very densely staining protoplasm, and its nuclei were evenly distributed through its whole interior (Figs. 6, 7, and 10), but with the migration of the antheridial nuclei into the conjugation-tube, the nuclei of the oogonium begin to migrate toward its centre (Fig. 14) where they become collected in the most typical cases in a very dense hollow sphere, the diameter of this sphere being about half that of the entire oogonium. Very frequently, however, instead of forming a sphere, the aggregation of nuclei may take the form of an irregular crescent either in the upper or lower half of the oogonium. In less frequent cases several masses of nuclei are formed instead of a single one.

The cytoplasm of the oogonium around this mass of nuclei has entirely changed its appearance. Instead of being charged with strongly staining substances it has become extremely tenuous and loosely spongy in its texture (Figs. 14, 15), so that the nuclei stand out much more sharply differentiated than before. The nuclei in this central mass are embedded in a cytoplasm which is essentially similar to that in the periphery of the oogonium. There has been an actual disappearance from the protoplasm of a considerable amount of densely staining substance at this stage, but its fate is by no means certain. The oogonium has also increased somewhat in size and its wall has become somewhat thicker; but whether these ordinary phenomena of growth are sufficient to account for the change in the density of the protoplasm must be left undetermined. After the oogonial nuclei have become aggregated in the centre of the oogonium the basal wall of the conjugation-tube breaks down (Figs. 15, 15 a), and the antheridial nuclei are admitted to the oogonium where they pass at once to the central mass of egg-nuclei and become mingled with them. Fig. 14 shows that there is no special differentiation of the protoplasm on either side of the basal wall of the conjugation-tube either before or at the time of its disintegration. There is no formation

of dense disks of fine granular cytoplasm such as were observed in connexion with the fusion of the tube with the antheridium. The receptive spot seems to be associated here as in the Alga egg with the bringing together and fusion of two cells which have not been in contact, and to be entirely unnecessary where the mere breaking down of the wall between two adjacent cells of the same structure is to be accomplished.

We must note here at once that the processes described are not at all of the nature of a secondary fertilization. The presence of the receptive spot at the apex of the conjugating tube shows that the latter is an organic part of the oogonium, and that it is the fusion of tube and antheridium which is homologous with the fusion of egg and sperm in *Oedogonium* or *Vaucheria*, while the breaking down of the basal wall of the tube is a later development necessitated by the formation of a conjugating tube on the oogonium. However, under the conditions found in *Pyronema* both fusions are necessary for fertilization, and the question is merely one of homologizing the processes here with those in oogonia, where no beak-like conjugating tube is present; and as already noted, from this standpoint there can be no question that the possession of a conjugating tube on the oogonium is a later and more specialized condition than that in the Algae named, and that the conjugating tube is merely a portion of the oogonium set apart for the conduction of the male nuclei to the egg-nuclei. The nuclei of the tube are therefore potential egg-nuclei, and their disintegration is physiologically analogous to the disintegration of potential eggs as nurse-cells, &c., which is seen in so many cases in the ovaries of animals.

We may conclude then that although we have two cell-fusions, i. e. first, the fusion of the antheridium with the tube, and second, the fusion of the compound cell so formed with the oogonium, these are not to be interpreted as two fertilizations. The contents of the tube-cell are entirely passive after the fusion-pore has been formed, the real and only



fusion of protoplasmic substances for purposes of reproduction being that between the antheridium and oogonium.

The passage of the antheridial nuclei through the tube into the oogonium, when once the way is open, is a rapid one. As has been seen already the tube is filled with antheridial nuclei before its basal wall is dissolved. The opening formed is larger than that between antheridium and tube, the entire basal wall of the tube being disintegrated. As a result there is an actual protoplasmic continuity between the male protoplasm in the tube and the egg-protoplasm. The two masses fuse at their point of contact after the tube wall has disappeared, so that the male nuclei enter the oogonium not like so many independent sperms, each piercing the plasma-membrane of the egg, but by simply migrating from the one cell-body to the other through a continuous mass of protoplasm. The entire structure, antheridium, tube, and oogonium, have in reality become for a time a single cell bounded by a single continuous plasma-membrane.

In passing into the oogonium, the male nuclei leave behind the bulk at least of the cytoplasm with which they have been associated. The cytoplasm of the antheridium has been carried to some extent into the conjugating tube, but for the most part it remains in the antheridium. The nuclei here, as in so many other cases, indicate their superior significance in sexual reproduction by leaving behind the cytoplasm which has hitherto been associated with them.

One conspicuous result of this non-participation of the cytoplasm in the act of fertilization is, that the antheridium and conjugating tube remain turgid and to the superficial view unchanged for a long period after the fusion has taken place. Tulasne observed this and remarks, as referred to above, that if the macrocyst and paracyst do influence each other mutually, this at least produces no notable change in their external appearance. As seen in sections the antheridium contains a series of large vacuoles in its central portion which were not present immediately before the migration of its nuclei (Figs. 15, 17, 20, and 21). The presence of these

vacuoles however does not constitute a very noticeable change as seen in the living cell, especially since in its earlier development the antheridium is decidedly vacuolar. It is interesting to see that the actual process and nature of fertilization in *Pyronema* could not have been discovered by the earlier observers, to whom the nuclei were invisible since it is practically the nuclei alone which pass over from the male to the female cell.

The number of male nuclei which enter the oogonium does not appear to be exactly the same as the number of egg nuclei to be fertilized. I have counted them in several cases and found upwards of two hundred in each sexual cell. It is difficult to see, since oogonium and antheridium are each developed from a multinucleated cell to start with, and there is no evidence subsequently of a parallel series of nuclear divisions in each, how exactly the same number could be provided in each case. As a rule when fertilization is accomplished by free motile male cells the latter largely outnumber the eggs. There is here no necessity for such excess, and the numbers, as I have pointed out, appear approximately equal. It will be seen however later that there is good evidence of slight inequality in the number on one side or the other, and that these supernumerary nuclei die without further development. It should be mentioned in this connexion that there is quite commonly an excess of antheridia. I have frequently found two antheridia applied to the surface of one oogonium, but only in one case have I found them in connexion with it (Fig. 21). In this case the conjugating tube was forked, sending a branch to each antheridium as the figure shows. Kihlmann describes a branching antheridium connected with two oogonia. The supernumerary antheridia are abundantly filled with nuclei and seem normal in every respect. They do not however develop further, and may be found very frequently at a late stage of development protruding from the base of the hypothecium with their contents quite unchanged, except for the aggregation of their nuclei at the apex of the cell. This is at a time when the

functional sexual cells are entirely emptied of their contents. They thus furnish very interesting evidence of the sexual nature of the fusions, in that they show that the cells concerned are incapable of further development if the fusion fails to occur.

In view of what is described below of the fusion of nuclei in the oogonium this kind of evidence seems hardly necessary. Still, in discussing conidial fusions in the Smuts, Brefeld (5) has insisted especially upon the inability to develop further without fusion as a final test of the sexual nature of cells, and in these supernumerary antheridia we have a most striking example of this particular sort. Ultimately the contents of these antheridia disorganize into an undifferentiated deep staining mass which then becomes a prey to Bacteria and disappears. Brefeld doubtless overestimates the value of this sort of evidence, as I have endeavoured to show in another connexion above.

How such waste of material comes to occur, when one might suppose the development of a single oogonium and antheridium together could be correlated from the beginning of the development of a fruiting cluster, is not so easy to understand. Such duplication of the antheridial branches never occurs in the Mildews so far as I have observed. It may be that this want of correlation is to be taken as indicating that the sexual cells arise from rather widely separated branches of a mycelium if not from branches arising from different mycelia.

The complete migration of the male nuclei into the oogonium represents the usual case; still, it is not very uncommon to find a few nuclei left behind either in the antheridium or the conjugating tube. The significance of this latter condition will be discussed later. After the migration of the male nuclei is complete a wall is built across the base of the conjugating tube, cutting off the oogonium once more as a single spherical cell. On the other hand the pore between the antheridium and conjugating tube remains permanently open and can be readily recognized in all the subsequent growth



of the ascus-fruit until the entire sexual apparatus collapses at a late stage in development. The fusion of the conjugating tube with the oogonium is thus relatively transitory when compared to its fusion with the antheridium, and herein doubtless is the reason why Tulasne and Kihlmann failed to discover the former fusion while perfectly familiar with the latter. The reason for rebuilding the wall in one case and not in the other is perfectly apparent in that the oogonium is to develop further, while the functional activity of the antheridium and conjugating tube is completed with the accomplishment of fertilization.

The male nuclei, as has been noted, on entering the oogonium mingle with the egg-nuclei in the more or less central group into which the latter have collected. The nuclei are indistinguishable in size, structure, and staining qualities, so that it is quite impossible to pick out a single nucleus at this stage and say with certainty whether it has come from the oogonium or antheridium originally. We have simply a mass of scores of nuclei which have been brought together in the manner just described. These nuclei fuse in pairs, while they are yet aggregated in the dense mass described above. Fig. 15 shows a section of this mass at a stage when many of the fusions are complete. Fig. 15 *a* shows a section from another oogonium in which most of the pairs have fused. The mass is so dense at this stage as to make the study of the individual nuclei in its centre quite difficult even in very thin sections, but at the margin of the mass, and in tangential sections from its surface numerous pairs of nuclei can be found in various stages of fusion. Parts of such tangential sections showing fusing nuclei in various stages and from different oogonia are shown in Figs. 16, 16 *a*, and 16 *b*. Fig. 16 is from an oogonium with a crescent-shaped mass of nuclei. The other two are from oogonia with spherical masses of the type shown in Figs. 15 and 15 *a*. Although the nuclei from the antheridium are not distinguishable in size or appearance from those already in the oogonium, still there seems no reason to doubt that such

fusing pairs consist in all cases of male and female pronuclei from the antheridium and the oogonium respectively. It is also impossible, as I have just mentioned, to determine with certainty the behaviour of the nuclei in the centre of these masses; still, the large number of cases of fusion that can be observed leaves little doubt that the rule of fusion in pairs holds for practically the whole mass.

It will be observed that these fusions are not necessarily simultaneous, some pairs being completely united while other nuclei are not yet arranged in pairs. In the oogonium from which Fig. 15 is taken, it is to be seen in other sections that a few nuclei have already begun to wander out into the young ascogenous hyphae, while the ascogenous hyphae of the oogonium, from which Fig. 15 *a* is taken, contain as yet no nuclei.

It is not to be assumed from my statement as to the density of this mass of nuclei in the oogonium that anything in the nature of a general fusion of the nuclei into a single nucleus occurs. It is perfectly certain, as seen from my Figs. 15 and 15 *a*, that throughout these masses individual nuclei of typical appearance can be made out with perfect certainty and at a slightly later stage, when the nuclei scatter after fusion, it is still further apparent that the mass consisted of perfectly distinct nuclei (Pl. XX, Figs. 17 *a, b*).

This aggregation of the nuclei at the time of fertilization seems to be simply a provision for the pairing of the male and female nuclei with the greatest certainty and dispatch. If the sexual nuclei are attracted to each other chemotactically, it is easy to see that unnecessary migrations of the male nuclei after attempts at multiple fusions are avoided by having the female nuclei in close proximity with each other, and in as small an area as possible. If the female nuclei were irregularly distributed it would be possible that isolated unpaired male and female nuclei might remain at opposite sides of the oogonium outside the spheres of their mutual chemotactic influence. *Pyronema* thus affords an interesting example of the fusion of multinucleated sexual cells and the

subsequent pairing of the male and female nuclei in the oogonium. The more general significance of these phenomena I shall discuss further on.

While the conjugating tube is still in open connexion with the oogonium, and in some cases even earlier than this, we find papillae budding out over the lower surface of the oogonium. The base of the oogonium is also enveloped at this stage with the vegetative branches which are to develop the hypothecium (Figs. 14, 15). These vegetative enveloping hyphae are not distributed evenly around the whole base of the oogonium. To understand their arrangement fully the study of more or less nearly horizontal sections through the rosettes of reproductive cells is necessary. Fig. 18 shows such a section at an earlier stage prior to fertilization, and from it a number of facts can be made out as to the relation of the oogonium and antheridium to each other, and also as to the relation of the pairs of sexual cells to each other in the cluster. This section shows three pairs, which is perhaps a fair average number for the form of the Fungus I have studied. It is seen at once that the oogonia are arranged in a triangle and that the antheridia are turned toward their outer surfaces. This is quite a universal rule in the cases examined. While the antheridium may originate at almost any point near the base of the oogonium, it almost without exception takes a path in its growth which brings it to that side of its oogonium which is turned away from the other oogonia of the group. It can be seen, further, that in one case almost the full length of the conjugating tube can be seen although the section is inclined at a considerable angle to the vertical, showing that the axis of growth of the oogonium and tube is not a vertical line but is inclined outward from the base of the median vertical axis of the group. The distribution of the hypothecial hyphae can here be readily seen, and it is interesting to note that the young vegetative filaments which are to form the hypothecium and paraphyses are all growing upward between the oogonia of the group so that the outer surfaces of the latter are left entirely un-



covered. This inequality in the distribution of the enveloping hyphae may continue through their entire development. As they become more numerous in most cases they gradually spread around the base of the oogonium for about one-third of its height, leaving still exposed a large oblique circular segment of its surface. As a result of this habit of growth we have in the mature fruit-body a series of these exposed oogonial surfaces bulging out around its base, enclosed by a fibrous network of the vegetative hyphae. Sections of the fruits of course show these exposed oogonial surfaces only when they lie in a plane cutting such an exposed surface in a radial direction (Fig. 23 to the left). In other cases the sections may show the oogonia entirely (Fig. 20), and in some cases equally enclosed on all sides. In old fruits the secondary mycelium, as described below, forms a more or less perfect envelope around all these structures.

The vegetative hyphae developing in connexion with each of the whole number of oogonia of a cluster, whatever it may be, become combined in a single apothecial fruit-body as has been observed by all the earlier investigators of *Pyronema*. Such an ascocarp has been called a compound apothecium or syncarpium as compared with the ascocarp of *Erysiphe* which develops from a single ascogonium. Very frequently the rosettes of sexual organs grow so close together that further combination occurs by contact of adjacent ascocarps so that crusts or turfs of considerable extent may be formed.

It is, however, by no means unusual to find single isolated pairs of sexual cells and from these equally perfect simple apothecia develop. These simple apothecia are not, however, symmetrically developed around the original axis of growth of the oogonium. The vegetative hyphae always develop on one side of the oogonium so that a portion of one side of the latter is exposed near the base of the hypothecium just as in the case of the compound fruits.

At an early stage (Fig. 14) the vegetative enveloping hyphae have enclosed the oogonium quite fully on all but a portion of its outer and upper surface. The hyphae are several layers

thick and into this mass the papillae mentioned above as forming on the basal region of the oogonium push out (Fig. 19). These papillae are the young ascogenous hyphae. On the free outer surface of the oogonium many of them arise at a higher level than the vegetative hyphae have reached, and grow out a short distance free and unenclosed (Figs. 15, 15 *a*, and 17). These branches contain no nuclei at first.

At the time when the ascogenous hyphae first appear, the nuclei are still massed in the centre of the oogonium. As soon, however, as the fusion of the majority of the pronuclei is complete the dense mass begins to loosen up. This is well shown in two sections taken from different parts of the same oogonium (Figs. 17 *a* and *b*). At this stage the nuclear fusions are practically all complete. The nuclei are rounded up and show no trace of the fusions by which they were formed except an increase in size in some cases.

From the completion of the fertilization stage onward, the oogonium may be known as an ascogonium. It enters upon a renewed stage of vegetative activity which was made possible as a result of fertilization. In *Pyronema*, as we have noted, the ascogenous hyphae spring directly from the oogonium without its first developing into a multicellular organ as in *Sphaerotheca* and *Erysiphe*. This is, however, a minor difference; the fact that there is a period of renewed growth beginning at the time of fertilization and leading to ascus-formation, justifies the retention in all cases of the term ascogonium for the body from which the ascogenous hyphae spring. The scattering of the nuclei in the ascogonium is very rapid, as evidenced by the appearance of fusion-nuclei in the young ascogenous hyphae (Figs. 17 *a* and *b*) which when they were only a trifle shorter contained none (Fig. 15 *a*). It is also very apparent at this stage that the massing of the nuclei in the centre of the oogonium was not at all in the nature of a fusion. As soon as the fusion in pairs is complete each nucleus appears with perfectly sharp contours in the loosening mass. The fusion-nuclei at this stage show much more conspicuously differentiated nucleoli than did the pro-

nuclei. The chromatin is also sharply differentiated in the form of strands or granules on the inner surface of the nuclear membrane, or distributed in the nuclear cavity (Figs. 17–20).

The ascogenous hyphae continue their growth and the ascogonium is rapidly emptied of its nuclei and protoplasm (Fig. 19). These ascogenous hyphae are extremely crooked and soon become septate. At first they may grow downward as it were to meet the developing vegetative hyphae among which they wind about in the most intricate fashion, as is only partly shown in Fig. 19. This preliminary downward growth would seem to indicate that in some degree the ascogenous hyphae anticipate in their development the vegetative paraphyses with which they are to be associated. It is probably true that the greater ease with which the ascogenous hyphae can push in among the vegetative hyphae at this early stage, more than compensates for any loss resulting from the detour they make in turning downward at first instead of growing directly upward toward the position of the future hymenium.

After the ascogenous and vegetative hyphae have become thoroughly interwoven, a rapid stretching upward of the whole mass ensues. In this growth the vegetative hyphae outstrip the reproductive ones and form at first a cone-shaped mass made up of their elongated slender, densely aggregated tips. These elongated upper extremities of the vegetative hyphae of the envelope are the young paraphyses. Their number is being constantly increased by the pushing in of new branches from below, and thus the cone-shaped outline is maintained. The ascogenous hyphae grow upward for a certain distance in company with the vegetative hyphae. Their growth upward then ceases and they spread out horizontally, forming a rather dense layer at the base of the cone of paraphyses which may be called, if we restrict the term somewhat from its usual significance, the sub-hymenial layer. This interwoven horizontal layer marks the base of the hymenium. It is a little below the level of the point of union of the conjugating tube and antheridium (Fig. 20).



The paraphyses also show a limited apical growth, and having reached a height above the layer of ascogenous hyphae about equal to one-half the total thickness of the hypothecium, they also cease to grow in length and remain as a densely packed mass of parallel slender hyphae containing numerous minute nuclei in a vacuolated protoplasmic mass. They are septate and of about equal diameter throughout their entire length.

The ascogenous hyphae follow such irregular paths from the ascogonium to the hymenium that it is impossible to follow any one of them continuously for any great distance in a single section. They appear in the sections at this stage cut transversely and longitudinally in fragments of various lengths. They can always be distinguished from the vegetative hyphae with which they are intertwined by the size of the nuclei which they contain (Figs. 19, 20). The fusion-nuclei contained in the ascogenous hyphae have always a diameter two or three times as great as that of the nuclei of the vegetative hyphae. This makes the recognition of the ascogenous hyphae an easy matter, so that it is possible to trace their distribution anywhere in the fruit-body, as will be seen from Fig. 20.

In the stages after fertilization, as the central mass of nuclei becomes more distributed and the number of nuclei in the ascogonium is reduced by their migration into the developing ascogenous hyphae, nuclei of smaller size are frequently found mingled with the larger fusion-nuclei (Figs. 17 and 20). In some cases these smaller nuclei can also be found in the ascogenous hyphae (Fig. 20 *a*). They are the size of the original nuclei of the oogonium and antheridium. Fig. 22 shows an ascogonium in a stage when the young asci are forming, a little older than the stage shown in Fig. 20. The contrast in size of the nuclei still in the ascogonium is seen to be very striking. I am of the opinion that the smaller nuclei in this case represent supernumerary oogonial nuclei which have not been fertilized and are incapable of further development. Their remaining unfertilized

may have been due to the numerical inequality between the nuclei of the oogonium and antheridium, the lack in this case being on the part of the antheridium. They would then represent unfertilized egg-nuclei. It is evident they are incapable of further development since they are never found in the sub-hymenial layer of the ascogenous hyphae or in the young asci. In some cases they may be found in cells of the ascogenous hyphae as noted above, but never very far removed from the ascogonium. I am of the opinion as I have said that these are oogonial nuclei. It is, however, quite possible that they may be antheridial nuclei which found no egg-nuclei with which to fuse. This latter hypothesis would harmonize better perhaps with the general tendency in plants and animals to produce a larger number of male cells than of eggs. It was, however, noted above that in some cases a few nuclei were found in the antheridium after fertilization is complete and this has led me to assume that probably when an excess of male nuclei is present they are left in the antheridium. The chemotactic or other stimulus which leads the male nuclei to migrate through the tube to the oogonium would in this case be assumed to have exhausted itself when a number of the latter equal to the number of egg-nuclei had reached the oogonium. Whether these small and apparently unpaired nuclei found in the oogonium are male or female pronuclei, they do not develop further, and this fact is suggestive as to the necessity for fertilization and the impossibility of purely vegetative or parthenogenetic development on the part of the sexual nuclei in *Pyronema*. Ultimately these nuclei in the ascogonium become flattened upon its wall in an extremely thin layer of cytoplasm which is always found in it until a late stage of development. In the old fruits the nuclei and cytoplasm both are disorganized and appear as more or less scattered deeply stained granules.

If we turn now to the development of the asci from the ascogenous hyphae of the sub-hymenial layer we shall find nothing essentially different from what I have already

described for *Peziza*, *Lachnea*, *Ascobolus*, and *Erysiphe*. The young asci of *Pyronema*, like those of the Mildews, contain regularly two nuclei. The origin of these nuclei can be readily traced in *Pyronema*. The ascus here is quite regularly an outgrowth of the penultimate cell of the ascogenous hypha which bears it. Dangeard (7) has described this method of origin quite fully for *Peziza vesiculosa*, though he still says that the ascus may in some cases be the product of the fusion of separate hyphae. When the ascus arises from a single hypha he finds in the latter at first a single nucleus. This divides into two nuclei. Later a second nucleus appears which also divides into two. Of these four nuclei two are destined to fuse in the young ascus, one remains in the apical cell, and one in the cell behind that which develops the ascus. I find in general the same phenomena in *Pyronema* though, as will be noted below, the division of the two nuclei always occurs simultaneously.

The tip of the ascogenous hypha pushing up among the paraphyses becomes recurved. In this hypha we have a period of nuclear division. It at first contains two nuclei which divide into four nuclei. The divisions of the two nuclei are exactly simultaneous as seen in Figs. 25-29. The spindles are so placed that a pair of nuclei are left in the bent part of the hypha, a third lies in the recurved tip, and the fourth lies farther back in the hypha just below the point at which it bends (Pl. XXI, Figs. 29, 30).

The recurved tip is then cut off by a cross-wall (Fig. 31), and a second wall is formed parallel to, or at a slight angle with the first, and generally at about the same level, cutting off the hypha just below the bend. The dome-shaped cell formed in this way at the bend in the hypha is to develop the ascus. It contains two nuclei, and from the position and orientation of the spindles in Fig. 29 it is seen at once that they are not sister nuclei, and are each derived from a different one of the original pair of nuclei in the tip of the hypha. Whether these latter nuclei are formed from a single one of the fusion-nuclei by division I have been unable to deter-



mine with certainty. The only nuclear divisions I have seen in the ascogenous hyphae of *Pyronema* are the simultaneous divisions in the hyphal tips just referred to. These divisions are very readily observed and can be found in great numbers. It would seem if the paired nuclei arise by division of a single fusion-nucleus, that the nuclear figures should be readily found, but I have been unable to discover them.

*Pyronema* presents in the development of its ascogenous hyphae unusually simple conditions. The apothecia are very small and the total number of asci produced is not greater than could be provided for in the manner described without increase in the number of nuclei beyond those which are fertilized in the oogonium. In larger forms like *Peziza*, where the apothecia continue to grow in diameter for a long time, the conditions may be very different, and nuclear multiplication in the hyphae may probably provide for the development of the successive series of asci formed. It is to be remembered, too, that several ascogonia in *Pyronema* contribute ascogenous hyphae to the same apothecium.

The fate of the sister nuclei to those enclosed in the ascus is not certain. Dangeard seems to consider that the apical recurved cell of the hypha shows no further growth, though he is not very explicit on this point. I have never found evidence of this apical cell developing further. I have found frequent cases where it had degenerated, and it is safe to assume that this is the rule. As to the fate of the fourth nucleus I have found a number of preparations which show that in *Pyronema*, at least in some cases, it also does not develop further. Fig. 31 shows that the fourth cell from the hyphal tip produces the second ascus, the third cell containing the nucleus in question remaining sterile. The second ascus is formed just as the first was, the fourth hyphal cell pushing out into a lateral branch whose tip becomes recurved, just as in the first case. In the case figured the nuclei in the second branch are two in number and are in the equatorial plate stage. If the same plan were carried further back it might be the fifth cell which would produce the next ascus, the sterile cell in this

case being the basal cell of the lateral branch bearing the second ascus. I doubt, however, whether such a series could be found. The ascogenous hyphae produce such numerous short branches in *Pyronema* that I have never been able to find more than two asci connected in the fashion shown in Fig. 31. Such a system is doubtless itself a lateral branch. The formation of the asci is, however, clearly basipetal, as has been pointed out many times, and this makes it still more improbable that the apical recurved cell develops further after the first ascus is formed.

In brief, then, of the four nuclei formed by the divisions of the two fusion-nuclei, two in the case described undergo no further development. The other two, lying in the young ascus, fuse to form the nucleus of the ascus. Such a pair of fusing nuclei are shown in Fig. 33. The nuclei become somewhat enlarged before fusion occurs, the dome-shaped cell in which they are enclosed at first pushes out at its apex into a club-shaped process, which grows rapidly in length and becomes the ascus, which is thus supported on two cells at its base (Figs. 31 and 32). The two nuclei lie side by side or one above the other in the young ascus when they fuse. They become slightly flattened upon each other, their membranes disappear at the point of contact, and they form thus a single nucleus which is first oblong and becomes gradually spherical or oval (Fig. 34). This behaviour of the nuclei at the time of the formation of the ascus has not been as yet satisfactorily explained. Dangeard's hypothesis, that the ascus is an oogonium, is certainly not established, and as it was developed by its author without any investigation of the origin of the ascocarp or knowledge of the existence of nuclear fusions in its early stages, it can hardly be considered as applying to *Pyronema*. I shall further discuss the question of the significance of these fusions later on.

The phenomena of nuclear division and spore-formation in the ascus of *Pyronema* differ only in details from what I have already described for a number of forms. As it seems worth while, however, to have a complete account of the develop-

ment of forms like *Pyronema*, which have become familiar through their use as types in the text-books, I shall give a description of the main stages in the development of the ascus and the ascospores.

In the young ascus the protoplasm is rather evenly distributed, and the nucleus lies a little above the middle point of the long axis (Fig. 23). As the ascus increases in size a special denser mass of protoplasm, which is to form the spores, is differentiated in its upper portion. In the mature ascus this denser protoplasm is bounded above and below by a concave surface, and the remainder of the ascus is filled by a very vacuolar foamy protoplasm (Figs. 39, 40). The nucleus lies at first near the base of this denser mass. It is a large conspicuous structure with a single bright red-stained nucleolus and an irregular chromatin net. Fig. 35 shows an equatorial plate stage of the division of this nucleus. It is to be noted that this primary nucleus does not tend to occupy the centre of the dense protoplasm in which it lies. The two secondary nuclei into which it divides are also not arranged symmetrically as they are in the ascus of *Lachnea* for example. In *Pyronema* these two nuclei generally lie close together in a line which cuts the long axis of the ascus at a considerable angle. They remain in this relative position while dividing. Figs. 37, 38 show equatorial plate and dispirem stages of this second division. The daughter nuclei are still connected by the remnants of the spindle-fibres which have been drawn out into a narrow strand in Fig. 38. In this figure two nucleoli appear in the cytoplasm in the position of the nuclei before their membranes were broken down.

The four nuclei resulting from the second division become arranged in a symmetrical row in the long axis of the ascus (Fig. 39). The spindles of the third division lie more or less transversely, so that the eight daughter nuclei formed are at first in an irregular double row, all of them within the mass of denser cytoplasm.

The daughter nuclei of the eight-nucleated stage as in *Lachnea* are completely reconstructed before the beaks are



formed. The polar asters are persistent after the last division as in other cases I have studied, and when the beak is formed on the nucleus the aster lies at its tip. The structures at this stage in *Pyronema* are quite small, but all the principal stages in the transformation of the aster into a plasma-membrane for the ascospore can be readily distinguished. Fig. 40 shows an exceedingly common stage in which the beak is formed, and the aster is apparently flattened somewhat against the wall of the ascus. In the case of three of the nuclei in this ascus the axis of the beak was so nearly vertical to the plane of the section that it is not represented in the figure. The nuclei are in the resting condition and fully developed. They contain a single nucleolus as a rule, and the chromatin is in the form of irregular granules and rods, or even threads. The dense mass of protoplasm in which the nuclei are lying is entirely without indication of its future differentiation into spore-plasm and epiplasm. At the upper end of the ascus above the region where the spores are to be formed it is loosely spongy and vacuolar in texture. The same condition is found in the ascus below the spore-bearing region, but is not shown in the figure.

Fig. 41 shows a later stage in the spore-formation also very common in *Pyronema*, though not so common in the forms hitherto studied. We have here a stage when the spores are about half surrounded by the folded back rays of the aster. The rays are already fused to a membrane about the point of the nuclear beak as shown by the slight plasmolysis in the case of the lowest of the forming spores in the Figure. In two cases here also the beak does not appear because it stands too nearly vertical to the plane of the section. In both these cases, however, portions of the forming plasma-membrane appear in the positions where they are to be expected partly encircling the nuclei. The amount of material in the asters and developing membranes in this ascus is plainly much greater than is present in the stage represented in Fig. 40, and indicates, as I have pointed out before (16) that new kinoplasmic material may be formed

during the process of cutting out the ascospore. Probably the nucleus with the beak is a centre for this metabolic activity, the kinoplasmic material being distributed from the point of the beak as I have endeavoured to show more fully elsewhere. The process of growth of the rays and their fusion into a membrane continues backward around the nucleus until the latter is entirely enclosed in a new bounding membrane, and the spore is thus completely delimited by the process of free cell-formation. Later, a wall is developed around the spore, which in *Pyronema* never becomes especially thickened or sculptured. The ripe spores remain uninucleate. Their germination I have not studied.

A section through a mature compound apothecium is shown in diagram by Fig. 23. The secondary mycelium shown at this stage begins its development as an outgrowth from the superficial cells of the hypothecium in stages a little older than that shown in Fig. 20. These filaments may arise from any one of the hyphal cells which is on the surface. They are extremely thick, at least twice the diameter of ordinary filaments, and extend downward and outward until they reach the substratum, where they mingle with the hyphae of the mycelium. Their existence is necessary for the support and attachment of the fruit-body, since it would otherwise have only the original thickened hyphae of the cluster which bore the sexual organs as a connexion with the substratum. Whether these appendages also serve to draw up water and food from the substratum is uncertain. Their protoplasm is vacuolated. They are sparingly septate, and their cells are multinucleate. They are plainly similar to the supporting hyphae I have already described for *Ascobolus* (16), and are probably homologous with the more highly differentiated appendages of the Mildews. As already noted, the apothecium of *Pyronema* is a compound structure built up of the ascogenous hyphae and vegetative tissue developed in connexion with several pairs of sexual organs. Fig. 23 represents two ascogonia at the base of the fruit-body. In such cases as this it is very plain that the main development of the asco-

genous hyphae and paraphyses from any one oogonium and its stalk is always to one side of the oogonium and never about it as a centre. As noted above, the several oogonia of a cluster always develop their branches on their adjacent sides as far as possible so that the centre of development of the apothecium is the centre of the cluster of reproductive organs. In this way symmetrical circular apothecia are developed similar in appearance in all respects to the fruit-body of *Ascobolus*, which develops from a single ascogonium.

Such irregular masses, as are shown in Fig. 98 of De Bary's text-book (13), I have never found arising from a single rosette, but by fusion of the fruit-bodies from a series of rosettes crusts can be formed of considerable size and extremely irregular outline.

When, as I have noted above, a single pair of sexual cells develops a fruit-body, it is always unsymmetrically placed with reference to the oogonium which lies exposed on one side of the hypothecium. This shows that the development of the ascogenous hyphae and paraphyses chiefly on one side of the oogonium is not merely a correlation of the formation of the compound fruit-body. The true explanation is probably that the oogonium is too large relatively to the size of the entire fruit to be properly enclosed by the hypothecium without distortion of the hymenium and the formation of a poorly developed point in its centre. Hence the oogonium is left at one side, and its branches with the vegetative hyphae develop a symmetrical, somewhat top-shaped apothecium.

*Pyronema* belongs to that group of the Discomycetes in which the hymenium has no peridium or lateral boundary of vegetative protective hyphal tissue around the hymenium. This condition is very well shown in sections (Figs. 20, 23), the paraphyses being seen to form the outermost boundary of the hymenium on its entire periphery. This character in *Pyronema* is probably correlated with the habit of producing compound apothecia and still larger crusts by fusion. It would be plainly a waste of material to attempt to delimit



by a vegetative layer the particular ascogenous branches that come from each pair of sexual organs.

As in the other forms studied, the ascogenous hyphae are relatively transitory structures in their basal portions, and maintain no permanent system of connexions for the transfer of food from the ascogonium to the developing asci. The ascogonium persists longer than do the ascogenous hyphae, but it is merely an empty sac. The ascogenous hyphae can hardly be found in an old fruit. They have simply collapsed from the pressure of the growing vegetative hyphae around them. In the sub-hymenial layer the tips of the ascogenous hyphae continue to grow and branch until the fertilized nuclei are all utilized in forming asci. The collapse of the ascogenous hyphae at a relatively early stage indicates that it would be quite difficult for the asci to be nourished through them from below. The development of the asci is relatively slow, and, as I have noted in other cases, all the conditions indicate that the asci get their nutriment through their walls from the adjacent paraphyses.

The fact that not all the asci develop simultaneously necessitates the continued development of paraphyses from the hypothecium to provide for the later-developing asci. A special type of hypha has been developed in *Pyronema* to provide these late-developing paraphyses. These hyphae arise from the basal cells of the cluster or the stalk-cells of the oogonia, and push up among the vegetative hyphae of the hypothecium. They then become divided by septa into short cells which immediately swell into large spherical or barrel-shaped vesicular reservoirs<sup>11</sup> which, as fast as they swell, are packed full of protoplasm with numerous nuclei. This protoplasm is unusually dense. The diameter of the reservoir cells is twice or three times that of the ordinary hyphae, and altogether they are very conspicuous objects (Figs. 23, 24). In free-hand sections not stained to show the nuclei one would very naturally pick out these hyphae for the ascogenous hyphae. It is easy to prove, however, that they contain only vegetative nuclei, and are ultimately de-

veloped above into paraphyses. I have pointed out above that Krabbe in his work on *Cladonia* may very likely have mistaken some form of storage hyphae, such as those I have described, for the ascogenous hyphae, and thus have been led to the false conclusion that ascogenous hyphae and paraphyses are ramifications of the same vegetative system. In stained microtome sections of *Pyronema* there is no necessity for such a mistake. The ascogenous hyphae develop much earlier than these swollen hyphae, and have largely disappeared in the lower part of the hypothecium when the latter appear. The nuclei also furnish an unfailing criterion, those of the ascogenous hyphae being two or three times the diameter of those in the vegetative hyphae. Furthermore, the ascogenous hyphae never appear as densely packed with protoplasm as do these vegetative cells.

The protoplasm of these swollen cells is finely differentiated in their central parts into a system of fibres which extend from one septum to the next (Fig. 24). These fibres are curved so that when taken together they form a strongly swollen barrel-shaped figure. This system of fibres is very prominent in the cells in question. Its interpretation is not easy. Perhaps the fibres represent paths along which materials are transported from one cell to the next. The piling up of protoplasm in these cells doubtless requires considerable transportation of foods from one cell to another, and this may possibly have led to the very conspicuous differentiation of these fibrous tracts. Whether there is actual protoplasmic continuity, or even protoplasmic contact, between these cells by means of pores in their septa I have not determined. Ultimately these vesicular cells become emptied of their protoplasm and appear as other hyphal cells of the hypothecium except for their much larger size.

#### GENERAL CONCLUSIONS.

If we interpret the phenomena I have described as occurring in the antheridium and oogonium, in the light of what is

known of sexual fertilization elsewhere, the conclusion is inevitable that we have here also a true sexual reproduction. Cells of separated origin fuse by means of the breaking down of cell-walls and the union of protoplasmic masses at the point of contact, and this protoplasmic union is followed later by a pairing of the nuclei. These facts are conclusive as to the homology of the processes here with the sexual processes in *Cystopus*, *Oedogonium*, *Nemalion*, *Batrachospermum*, &c., and it seems hardly necessary to discuss the assertion that such fusions are the same as those which occur between the ordinary vegetative hyphae of a mycelium. I have discussed the significance of these latter fusions elsewhere (17), but it only needs a moment's consideration to show, that they have no direct relation with the sexual conjugation I have above described. These mycelial fusions are not between specially differentiated hyphal cells. They occur at no definite point in the life-history of the Fungus, and lead to no specific fruit-formation. Indeed the comparison is so shallow and unenlightening, even on the basis of Tulasne's original description of the fusion in *Pyronema*, that it is hardly worthy of a detailed consideration.

Van Tieghem (37, p. 1166, note) has brought forward the argument that the methods of sexual reproduction, as described for the Lichens, Mildews, *Eurotium*, *Pyronema*, &c., are too varied to be found in related genera of such a group as the Ascomycetes. Such *a priori* arguments are of very little value in connexion with a subject so little investigated as the sexuality of the Ascomycetes, in which the number of forms whose ascus-fruit development has really been thoroughly investigated is so small in comparison with those in which, because of their small size or the difficulty in separating the Fungus from its substratum, &c., the course of this development in its earlier stages is largely unknown. For the present we shall have to admit that whether it is reasonable or not there are in the true Ascomycetes at least such types of sexual apparatus as have been described for the Laboulbeniaceae, the Lichens, the Mildews, and *Pyronema*. It is



not improbable that further investigation will show connecting links between these forms, but that is entirely a matter for investigation rather than speculation.

Van Tieghem also suggests (37, p. 1142) that the swollen oogonium and the antheridium in *Pyronema* are only reservoirs of stored food material brought together by the hyphae at certain points in anticipation of unusual outlay in rapid growth during fruit-formation. In view of the behaviour of the nuclei of these cells as I have described it above this interpretation becomes quite inadequate. Female sexual cells are commonly well stored with reserve food-materials, but no one will seriously contend that they are to be explained merely as reservoirs for storage.

Van Tieghem's hypothesis was without foundation on the evidence which had already been submitted by Kihlmann, who showed that only the ascogenous hyphae are developed from the specially swollen cell, while the whole hypothecium and paraphyses arise from mycelial cells below the sexual apparatus. Bauke had already pointed out that the asci are nourished from the paraphyses, and all observers had agreed that the ascogonium withers at a relatively early stage. Surely it was unreasonable to assume that an organ specially developed as a reservoir of nutrition should fail midway in the completion of its function, not even serving in its later stages as an avenue for the conduction of new food-materials to the asci. It seems not too much to claim that the opponents of sexuality in such forms as *Pyronema* seem to have been bent on overlooking the more obvious facts in the interest of ingenious but unreasonable hypotheses.

The investigation of the sexuality of the Fungi has become involved to an unusual degree in the personal animosities of rival investigators, and as a result De Bary's pre-eminence, as the first who attained the technical skill necessary for grappling with the problem, has been too little recognized. De Bary and his pupils brought together a greater mass of accurate and detailed observations on the life-histories of the forms in question than any or all of the opponents

of his views can muster. Opposition to his view of the nature of the ascus-fruit in the forms he studied has not been based to any appreciable extent on detailed investigations of the development of these structures in the same or in related forms, but rather on a general scepticism as to the accuracy of De Bary's observations, and on vague and fanciful hypotheses such as those referred to above. De Bary's generalization, as to the sexuality of all the Ascomycetes from the relatively few forms he had studied has been much criticized as based on too few observations. Still it is to be noted that no successful attempt has ever been made to subdivide the group in question. Regarding the group as a phylogenetic unit it was and is quite justifiable to assume that such similar structures as the ascus-fruits must be homologous, and if in one case their initial organs are a sexual apparatus, whether functional or not, the assumption is, until the contrary is proved, that the same will be true for the other members of the group, all due allowance being made for a possible suppression or further development of any particular stage in special cases. So long as there is no evidence from other sources on which to subdivide the group there is the strongest probability that the ascus-fruits of all Ascomycetes are morphologically equivalent to the ascus-fruits of *Sphaerotheca*, *Pyronema*, and the Lichens.

The opponents of De Bary's views, apart from Dangeard, who so far stands alone, have shown no less certainty of the unity of the group than did De Bary, and have concluded that the entire group is without sexual reproduction, because they believe functional sexual organs are lacking in some specific cases, although even in these the nuclear phenomena have not been yet investigated. Whether it may not be found after all that the Ascomycetes are a polyphyletic group is still an open question, but at present, as heretofore, the evidence seems rather to point the other way. For the formation of sub-groups and the determination of their relationships the cell-structures of such forms as *Eurotium*, *Xylaria*, *Claviceps*, *Pleospora*, &c., in addition to those

already known, must be thoroughly investigated and compared.

The motive to such investigations lies, however, not so much in the desire to further pile up evidence on the question of the sexuality of the initial cells of the different ascus-fruits as in the hope that thereby further light may be thrown on the more general questions as to the structure and functions of the cell. The peculiar types of cell-division, fertilization, &c., found in the group may be expected to afford useful data for the solution of general physiological problems. One of the most interesting features in the reproduction of *Pyronema* is the fact that we have in it a case of the fusion of multinucleated sexual cells. The hyphal cells of *Pyronema* are regularly multinucleate, and, as has been shown, the oogonia and antheridia contain a large and variable number of nuclei at all stages in their development. In the fusion of these cells the nuclei fuse in pairs so that the general result, so far as the nuclei are concerned, does not differ from what is found in sexual fusions ordinarily.

An interesting question arises, however, as to the nature of such multinucleated sexual cells. Is the oogonium for instance to be regarded as a mass of oogonia whose cell walls have been obliterated or have never existed, or is it to be regarded as a single cell? Is a 'coenocyte' a cell or a tissue? It is not merely a question of names, since from both the morphological and physiological standpoints our conceptions of the cell and of the tissue differ very widely, and it is worth while to determine, if possible, which of these types the 'coenocyte' resembles more nearly. The suggestion of the energide doctrine by Sachs has been taken up uncritically and carried too far by many. The cell is not necessarily a uninucleated mass of protoplasm. Whether the conception of the cell should be so extended as to include such differentiated structures as *Caulerpa* and *Bryopsis* is perhaps not so plain. Still the difficulties involved in regarding them as cells are not due to the fact that they are multinucleated. The differentiation shown by these plants is in the cytoplasm and



not, so far as we know, in the nuclei, which are all at least potentially equivalent in their capacity to transmit the hereditary characters of the organism or to influence the metabolism, since so far as known any of these nuclei may be used to form a reproductive spore, and by the streaming of the cytoplasm they are carried successively to rhizoid, stem, and leaf.

These organisms would be quite as difficult to include in the category of cells, if they were uninucleate, if that were conceivable. They represent the extremes of differentiation yet discovered in a structure enclosed by a common cell wall and plasma-membrane, but it is quite as difficult to consider them from the standpoint of tissues since in their metabolism and irritability, they are by no means compounds made up of equal and co-ordinate units. On the whole it does less violence to the facts to class them as cells than in any other category yet proposed. There is nothing in our conception of a cell that is negatived by the assumption of the differentiation of its parts into organs of nutrition, organs for attachment, reproduction, &c.

In the case of all such multinucleated cells as are combined in filaments or other structures the interpretation is much easier. The red Algae afford exceedingly interesting evidence against the possibility of separating uninucleated from multinucleated cells, since in immediate connexion with each other in the same thallus we have vegetative cells that are regularly uninucleated, and others that are just as regularly multinucleated. It is plainly doing violence to the most obvious relationships to call the one type a cell and the other a tissue.

The multinucleated vegetative cells and sexual cells of *Pyronema* fall in this same class, and if we compare in detail these coenocytic cells with uninucleated cells and with tissues composed of the latter, it will be seen that the coenocyte is a single cell in all its essential properties and not a compound. Anatomically and morphologically the cell is a mass of protoplasm enclosed by a continuous semi-permeable and

irritable membrane, and from this standpoint it may be one- or many-nucleated. That there is a certain unity in a mass of tissue such as a palisade parenchyma made up of cells combined in a leaf for the performance of a definite function is not to be questioned, but this unity is achieved by the combination of the activities of a number of independent co-ordinate individuals. Each cell in the tissue receives its own nutrition and throws off its own wastes, and each receives and reacts to stimuli from its environment.

The continuous plasma-membrane enclosing a coenocyte is plainly in its relation to the other cell-contents to be compared with the same structure in a uninucleated cell rather than with the aggregate of membranes, which bound off a mass of tissue from its environment, and the cells of the tissue from each other. And it is this bounding layer which through its properties as a semi-permeable membrane regulates the income and outgo and thus the nutrition of the cell, and also, according to Noll (26) at least, determines its reactions to external stimuli.

The sum of the reactions in a mass of tissue-cells may be in harmony and serve some common end for the organism, but they are none the less independent in origin and in their accomplishment in that they are co-ordinated. In the case of a multinucleated cell we have no evidence that either the process of taking in food or the reception of stimuli is in anything different from what it is in the case of a uninucleated cell—except perhaps in the amount of food taken in—the multinucleated cell being frequently proportionally larger.

The motions of a multinucleated plasmodium compared with those of a uninucleated amoeba show no indication of being compounded from the motions of independent units. So far as can be judged from its amoeboid and streaming movements the plasmodium is a unit in the same sense as is the amoeba.

Schenk (30) has presented evidence that the katabolic processes by which energy is liberated are functions of protoplasm independently of its organization into cells, and that

similarly contractility and irritability are manifested by protoplasm independently of the aggregation of its parts in cells. A bit of an amoeba cut off from the parent cell and not containing any portion of nuclear material still responds to stimuli, is contractile, and creeps about in apparently normal fashion. It must be concluded that the liberation of energy for motion and the response to external stimuli is independent of any interrelation between nucleus and cytoplasm, and in the manifestation of all such phenomena the uninucleated and multinucleated cells would be at least on an equal footing.

The uninucleated swarm-spore of *Oedogonium* acts no more as a unit in its reactions to light than does the multinucleated swarm-spore of *Vaucheria*. So far as we know the pollen-tube with its vegetative nucleus responds to chemical stimuli just as does the multinucleated hypha of *Mucor*. The uninucleated conidiophore of *Basidiobolus* orients itself with reference to light-stimuli just as does the multinucleated sporangiophore of *Pilobolus*.

The metabolism inside the cell in that phase in which food is changed into organized material is doubtless dependent, as Schenk points out, on the interaction of nucleus and cytoplasm. The metabolism of the multinucleated cell is probably carried on in connexion with many centres instead of a single one, but in this respect the conditions here would not be strikingly different from what they are in such cells as are found in the spinning-glands of insects in which the single nucleus is branched so as to extend into all parts of the cell interior (24). These lining elements of spinning-glands cannot certainly be excluded from the category of cells, and, so far as the relations of their internal metabolism to their nuclei are concerned, they cannot be very different from multinucleated cell structures.

The statement has often been made that the *Vaucheria* filament is a mass of cells which merely lack their partition-walls. There is, however, no evidence that the reception of food in *Vaucheria*, its distribution to various parts of the



filament, and use in furnishing energy for carrying out heliotropic reactions and building cell walls is any different than the corresponding processes in a pollen-tube or a uninucleated cell of *Spirogyra*. It is probable, as noted above, that the nuclei are centres for the internal metabolism of the cell, but there is no evidence that they influence its absorption of food-materials by osmosis and its excretion of wastes any differently in the uninucleated than in the multinucleated condition. It is quite conceivable that any cell of the leaf-mesophyll should perform all its starch-building functions in entire independence of its neighbours, even to the extent of consuming its own product and excreting the wastes, but we cannot conceive any particular uninucleated area (energide) in a *Vaucheria* filament acting in such perfect independence of neighbouring areas, simply because in the latter case the uninucleated area is not an osmotic unit, but only a part of a larger osmotic system bounded by a single plasma-membrane. The mesophyll-cell in these respects is comparable to the entire *Vaucheria* filament.

In *Volvox* the income and outgo can be considered for each individual of the colony separately, and can vary in each according to the individual peculiarities of the cells, while for the so-called energides of *Botrydium* no such conception is possible. The income and outgo of the whole mass is regulated by a common plasma-membrane. Even assuming that the strands of protoplasm which extend between the neighbouring cells in many tissues really represent continuity and not merely contact of the protoplasm of the respective cells, the case would not be materially altered, since the extreme tenuousness of such strands in most cases would prevent their serving as channels for any rapid interchange of food-materials, as has been pointed out by Pfeffer (28).

Further, the processes of nuclear and cell-division are independent of each other as is most clearly shown in *Cladophora*, for the lower plants, and in certain types of endosperm-formation in the Phanerogams. From this standpoint we can still further sharpen our distinction between cells and tissues

by noting that it is only the division of the cell-body as a whole which leads to tissue-formation. Nuclear division may occur without cell-division and consequent tissue-formation, and in the case of sporangia, asci, &c., nuclear division may be followed by cell-division without tissue-formation. Nuclear division without cell-division gives opportunity for growth of the cell-body but is not tissue-formation.

In reproduction the relations are perhaps not so clear. Multinucleated cells reproduce by much the same type of division in *Cladophora* as do uninucleated cells in the conidiophores of *Erysiphe*. For sexual reproduction multinucleated cells may return to the uninucleated condition as is presumably the case in the motile gametes of *Botrydium* and *Acetabularia*. But that this return to the uninucleated condition is not necessary for sexual reproduction is shown by the case of *Pyronema*.

In this connexion it is interesting to note that Stevens (33) has recently described the fertilization in *Albugo* (*Cystopus*) *Bliti* as consisting in the fusion of multinucleated sex-cells. His account is entirely opposed to the results obtained by Wager (39) and Berlese (3) on other species of the same or closely related genera. If it is true that we have multinuclear fusions in the sexual cells of one species, and fusion of a single pair of sexual nuclei in those of another, it is further evidence of the close similarity between uninucleated and multinucleated cells. Stevens seems to take the view that his oospheres and antheridia are the equivalents of tissues, since he designates them respectively as compound oospheres and compound antheridia as compared with uninucleated cells with the same function. He does not, however, discuss the question specifically, and his use of the term compound is not entirely clear in this connexion. For example, he does not call attention to the multinucleated conidia of the *Albugo* as compound conidia, though from analogy he might be expected to do so.

In asexual reproduction by swarm-spores we have both types of cells; for example, uninucleated swarm-spores in

*Oedogonium* and multinucleated swarm-spores in *Vaucheria*. As asexual reproductive bodies there is no reason for putting the swarm-spores of *Oedogonium* and *Vaucheria* in separate categories so far as their function is concerned.

In the sexual reproduction of *Pyronema*, however, we have a combination of conditions in that the cells first fuse as individual units, and the nuclei then also fuse in pairs. The oogonium and the antheridium act as units in accomplishing the union and fusion of their respective protoplasmic bodies. There is a single receptive spot at the end of the trichogyne which functions for the entire contents of the oogonium. The oogonium of *Pyronema* in this respect is to be compared directly with the oogonium of *Oedogonium*. In the attraction which leads to the fusion of the antheridium and conjugating tube both organs may play a part, but they certainly act in this case in a fashion not to be distinguished so far as their nuclear content is concerned from that which brings together the conjugating tubes from the uninucleated cells of *Spirogyra*. The fact that there is no rounding up of an oosphere in the oogonium of *Pyronema* is plainly a secondary phenomenon. In the growth and metabolic processes leading to the differentiation of these cells as sexual organs, there is nothing to distinguish them from uninucleated eggs or sperm-cells. In the nuclear fusions, however, the nuclei, with perhaps a trace of cytoplasm for each, are the units which unite in pairs.

This apparent return in so fundamental a process as sexual fusion to a condition in which the single nuclei act independently may suggest that the original primitive cell was uninucleated. The condition of the multinucleated protoplasmic mass as found in the Siphoneae, for example, would then be regarded as a later modified development. If, however, as has been maintained by several authors<sup>1</sup>, the chromatin is distributed in the form of granules in the cell of a blue-green Alga, this fact must be regarded as evidence that the multinucleated cell may represent quite as primitive a condition as does the uninucleated cell. The behaviour of

<sup>1</sup> See Fischer, *Cyanophyceen und Bacterien*, p. 35. Jena, 1897.



the nuclei in multinucleated gametes is not to be regarded as conclusive evidence on this point, even if it bears at all upon it. It is merely a proof that the nuclei are the structures especially entrusted with the function of sexual reproduction, and certainly is not good ground for deciding that multinucleated protoplasmic masses are to be considered as tissues rather than cells. We must rather enlarge our conception of the cell from the morphological standpoint to include all protoplasmic masses enclosed by a continuous plasma-membrane. Physiologically it is the unit in nutrition, growth, irritability, and asexual reproduction, whether one- or many-nucleated. In sexual reproduction, on the other hand, the function of transmitting hereditary characters is probably to be attributed largely to the nucleus as an independent organ.

The significance of multinucleated cells in the plant-economy is doubtless different under different conditions. After the cell has reached a certain size further growth makes distribution of the nuclear material desirable in order that the nuclear activities may be represented equally in all its parts. In the hyphae of *Sphaerotheca*, on the other hand, whose cells contain one to several nuclei, this inequality and the multinucleated condition itself may be merely the result of the fact that a perfect correlation between the processes of nuclear and cell-division has not yet been attained.

In the sexual cells of *Pyronema*, as I have described them above, the multinucleated condition is plainly an advantage in insuring the simultaneous fertilization of large numbers of nuclei, and in the motility which is thus secured for relatively large masses of protoplasm. If the oogonium, for example, were uninucleated, a period of nuclear division would have to be interposed between fertilization and ascospore formation which is probably a much less favourable time for such processes than that prior to fertilization, when the numerous nuclei of the oogonium, as we have it, are produced. The conditions in *Pyronema* as compared with those in *Nemalion*, for example, may be regarded as intended to transfer the work of producing the numerous nuclei to be used in spore-

production from the period after, to that just preceding fertilization.

If we attempt to compare the sexual organs and the ascus-fruit of *Pyronema* with the reproductive organs in other Fungi and Algae, the fundamental resemblance in the process here to what we find in the Lichens as described by Stahl and other authors noted above, is at once apparent. De Bary (13) has already discussed this resemblance fully but without a knowledge of the behaviour of the contents of the sexual cells in the fertilization of *Pyronema*. The fact that the basal wall of the conjugating tube is broken down for fertilization strengthens the resemblance between this organ and the trichogyne of the Lichens. To be sure, the actual fertilization has not yet been observed in any Lichen, still the morphological significance of the organs concerned is established beyond question. That the trichogyne should be a multicellular hypha is remarkable; but the fact that the entire mass of antheridial nuclei does migrate through a connecting tube to the oogonium in the case of *Pyronema*, makes it less difficult to assume the correctness of Stahl's interpretation of the structures in the Lichens. The fertilization in the Lichens consists presumably in the fusion of a single pair of sexual nuclei, but this cannot affect the question of the homology of the parts as I think I have shown above.

The resemblance between *Pyronema* and the red Algae is certainly very striking. The tubular trichogyne which is simply a prolongation of the oogonium of *Nemalion* or *Batrachospermum* is strikingly similar to the conjugating tube in *Pyronema*. Davis (10) has endeavoured to show that the trichogyne of *Batrachospermum* is a distinct cell. His results are opposed by Schmidle (31) and Osterhout (27) who deny the existence of a nucleus in the trichogyne. But in any case the homologies would not be affected. The trichogyne in *Pyronema* is an outgrowth of the oogonium, and it would make little difference in its morphological significance whether a cross-wall were put in between the two or not, though the development of this wall is of considerable functional impor-

tance. With its one-celled trichogyne *Pyronema* forms an interesting link between the Florideae, where the trichogyne is a mere beak on the oogonium, and the Lichens, where it is a row of cells.

In the discussion of the question as to whether the tendency to dioecism is a sufficient explanation for the appearance of spermatia in the place of attached antheridial cells, there is little evidence to be added to that presented by De Bary. As to the general similarity of the apothecium of *Pyronema* and the cystocarp or favella of the red Algae there can be no question. In each we have a fertilized cell which by vegetative growth develops a mass of spores, in the one case ascospores, in the other carpospores. That in the one case the spores are produced by free cell-formation, while in the other they are budded off from the surface of a placental cell or cells does not affect the resemblance so far as its general features are concerned. Whether the vegetative growth of the fertilized cell is to be interpreted as an alternation of generations in either or both cases remains to be settled by a more accurate account of the chromosome-number in the two stages. That there is no oosphere rounded up and set free in an oogonium in either case cannot be regarded as a fact of very fundamental significance. The fertilized egg of the Moss or Liverwort remains in parasitic relationship with the mother plant through its whole development. In *Sphaerotheca*, for example, we need only consider that the parasitic relationship is more perfect, so that the egg is never set free from actual vegetative union with the parent plant. However the nature of the growth directly from the egg may be interpreted, there is no question that the protective hyphal envelopes developed around the ascogonium and asci are nothing but a further outgrowth of the sexual mycelium comparable to the development of the archegonium and calyptra in the Moss.

After what has been said of the relationship of *Pyronema* and the red Algae it will be seen at once that the connexion of the former with the Laboulbeniaceae must be both inter-



esting and suggestive. Thaxter (34, p. 253) considers that the Laboulbeniaceae may stand between *Sphaerotheca* and the red Algae. *Pyronema*, in the character of its trichogyne, stands closer to the red Algae than do the Laboulbeniaceae, but in its attached antheridial cell it is more like *Sphaerotheca*. Thaxter (34, p. 225) has described a very interesting series of modifications in the development of the trichogyne in the Laboulbeniaceae. In *Stigmatomyces* it is a single, one-nucleated cell without branches. In *Peyritschella*, *Amorphomyces*, and other genera, the one-celled trichogyne has branches shorter or longer, and more or less numerous. Other forms have multicellular trichogynes which may be simple or very abundantly branched (*Teratomyces*). The extremities may be spirally twisted as in *Compsomyces*. In all cases the trichogyne is borne upon a special trichophoric cell which is thus interposed between it and the carpogonium which is to be fertilized. The conjugating apparatus is seen thus to consist of two independent cells in the simplest Laboulbeniaceae as compared with one cell in *Pyronema* (the trichogyne), and a simple tubular prolongation of the egg-cell in the red Algae such as *Nemalion* and *Batrachospermum*. Such forms as *Teratomyces* with multicellular branching trichogynes represent still more differentiated types in this particular than the Lichens, where it also seems probable that a trichophoric cell is interposed between the carpogonium proper and the trichogyne.

The discovery of these extremely complex multicellular trichogynes of the Laboulbeniaceae, whose function as conjugating organs cannot be questioned, and which are connected by forms of all stages of complexity with the simple one-nucleated trichogyne of the *Stigmatomyces* type, certainly removes all doubt as to the existence of structures which must be considered morphologically as multicellular conjugating tubes. And the account of the breaking down of the walls and the migration of the male nuclei through the trichogyne of *Pyronema* given above furnishes the positive evidence that such conjugating tubes are the channels through which male pronuclei are conveyed to the egg-nuclei with which they

are to fuse. The Lichen trichogyne is made up of many cells, while that of *Pyronema* is a single cell, but if two walls can be broken down to permit the passage of the male nuclei there is no reason why a larger number may not be. Under conditions which necessitate a longer trichogyne such as we find in the Lichens it is entirely natural that it should be a multicellular rather than a unicellular structure.

Arranged according to the degree of complexity of the trichogyne we should have a series as follows: *Batrachospermum* with the trichogyne a mere tubular outgrowth of the oogonium, *Pyronema* with this tubular outgrowth cut off by a septum so as to form a cell, *Stigmatomyces* with a second cell cut off as a trichophore at the base of the trichogyne, and *Collema* and other Lichens with the trichogyne divided by further septa into a row of cells, while the branched multicellular trichogynes of *Teratomyces* and *Compsomyces* afford examples of still greater complexity of development in this organ.

Such a series of course cannot be regarded as showing the relationships of the different forms in the order indicated. It merely shows the possibility of such a series of forms having existed in the ancestry of the Lichen, and thus does away to a large degree with the difficulty which has been felt over the conception that such a multicellular hypha as the Lichen trichogyne could serve as a conjugating organ.

Thaxter has made the very interesting observation that in some cases the trichogyne grows down at first toward the antheridium so as to seek out as it were the non-motile male cell and thus insure fertilization. In the genus *Zoidiomyces* the trichogyne grows down at first and receives the antherozoid on its tip when it turns and grows upward again into its apparently more normal position. This reversal of the ordinary relations of the sexes in the act of fertilization in which the egg-cell supplies an organ for seeking out the male cell is exactly what was observed to a less degree by Tulasne in *Pyronema*. The egg thus ceases to be a merely passive receptive cell when it has developed a trichogyne. And it

may be that the increased certainty of fertilization assured in this fashion is the condition which has enabled the plants possessed of trichogynes to dispense with motility in their male cells.

While it may be regarded as established that the general similarity between the sexual reproduction in the Ascomycetes and the red Algae as pointed out by De Bary (12, pp. 86-88) exists, still in my opinion it is of no special value at this time to attempt to establish, from the forms which have been worked out, just how an evolutionary transition may have developed the Ascomycetes from the Algae. More forms must be investigated, and then at best the proof will probably be inconclusive. It is plain that the Ascomycetes resemble the red Algae more than they do the lower Fungi<sup>1</sup>, but whether these resemblances are a result of blood relationship, or are merely due to that similarity in the chemical constitution of the protoplasm of different organisms, which under similar conditions enables it to develop structures nearly related in appearance out of rudiments which may be extremely diverse, is likely to remain a puzzling question.

I have shown elsewhere that there is no sufficient evidence that the sporangium of the Moulds has been evolved into the ascus of the higher Fungi, and that hence any classification which connects the two groups on the basis of an assumed relationship between these structures is purely artificial and formal. On the other hand there is as yet no direct evidence of the transformation of the cystocarp with its carpospores into the ascocarp with its ascospores formed by free cell-formation, and hence the attempt to form a natural classification in which the one group is regarded as representing the source of the other is premature at least. For the present we must be content to allow the Ascomycetes to stand alone, with the hope that the thorough investigation of all the types in the group itself as well as the forms of

<sup>1</sup> De Bary's (12, pp. 109-119) view of the relationship of *Sphaerotheca* to the Peronosporae was based on a want of knowledge of the structure of the mature ascogonium in the former and need not be further discussed.



doubtful relationship to the main series may bring further evidence as to its phylogeny.

If we turn now to problems of relationship inside the group itself, and compare the sexual apparatus as we find it in *Pyronema* and *Sphaerotheca*, we are confronted by some marked differences in detail. A conjugation-tube or trichogyne is entirely wanting in *Sphaerotheca*. A further difference lies in the fact that in *Pyronema* the oogonium functions without further growth as an ascogonium. All the fertilized nuclei pass into ascogenous hyphae and may reach the asci, while in *Sphaerotheca* the single fertilized egg develops a row of cells of which only one becomes an ascus, while the others undergo degeneration. There is nothing in *Pyronema* to correspond to this growth of an ascogonium from the fertilized egg, and in this respect it seems to represent a simpler condition than *Sphaerotheca*. Also the fact that the entire protoplasmic mass of the oogonium, except in the case of unfertilized nuclei noted above, passes out into the ascogenous hyphae seems perhaps to indicate a simpler condition. It is perhaps analogous to the setting free of the egg-protoplasm from the oogonial cell wall which we find in those oogonia which produce regularly one or more free oospheres in their interior.

It is possible again that the ascogonium in *Sphaerotheca* and *Erysiphe* is in reality comparable to a single ascogenous hypha in *Pyronema*, in which case the two types could be conceived as more nearly related. Such comparisons, however, may very well be deceptive, since it has been shown many times that resemblance in form may be found where no genetic relationship between the parts or organisms compared can be assumed.

So far, however, as the trichogyne is concerned, we can readily imagine the development of the type in *Sphaerotheca* into that in *Pyronema*. The oogonium would need only to develop a conjugating beak such as is seen in *Coleochaete*, and this could be readily conceived as gaining final differentiation as a cell by the putting in of the partition-

wall at its base. Such a change as this would be a very natural correlation of the multinucleated condition and consequent enlargement of the antheridial cell which we find in *Pyronema*. It is to be remembered that the long club-shaped antheridium of the latter is to be compared with the antheridial cell of *Sphaerotheca*, not with the entire male branch of the latter. It is easy to see that a cell containing the mass of nuclei present in the antheridium of *Pyronema* could hardly be perched upon the apex of the oogonium as is the small antheridial cell of *Sphaerotheca*. We can readily conceive, then, the development of the conjugating tube as a means of overcoming this difficulty. Still I am inclined to believe that in reality the reverse process has taken place, and that the sexual apparatus with the trichogyne represents the more primitive type for the Ascomycetes.

*Sphaerotheca* and the Erysipheae, as I have already pointed out, may well be considered as a highly specialized group because of their parasitic habit and haustoria and their complex appendages, as compared with the relatively simple secondary mycelia of *Ascobolus* and *Pyronema*.

In view of the difference between the sexual apparatus in the Erysipheae and that in *Pyronema*, it is very interesting to note that *Ascobolus*, in the structure of its fully developed ascogonium, while superficially it is more like *Sphaerotheca* than *Pyronema* in one most important feature, is much more closely related to the latter. While in *Sphaerotheca* and *Erysiphe* only one cell of the ascogonium has its contents utilized in the formation of asci, the entire contents of the ascogonium of *Ascobolus* are emptied by means of the pores in its septa into the cell from which the ascogenous hyphae spring, and thus are utilized in ascus-formation. Similarly, as we have seen above, the entire contents of the ascogonium of *Pyronema* are utilized in ascus-formation. In view of these facts it would be extremely interesting to know exactly the nature of the earliest stages in the development of the ascogonium in *Ascobolus*.

In the Laboulbeniaceae, as Thaxter shows (34), this differ-

entiation of the product of the egg has taken place in all cases. The carpogonium regularly divides into three cells, of which only the central one produces asci; and in some cases this central cell cuts off a so-called secondary stalk before proceeding to the development of asci. In the red Algae, too, the egg regularly produces a certain amount of sterile tissue in addition to the carpospores, so that from this standpoint the problem of the relationships of the various forms becomes a very complicated one.

Further, *Eurotium*, as described by De Bary, shows a very close relationship to *Pyronema* in the structure of the sexual apparatus (12), and also an interesting resemblance to the Lichens. Indeed, *Eurotium* forms a very good intermediate type between *Pyronema* and the Lichens in many respects. In it we have a coiled female branch made up of several cells, the upper one of which conjugates with an antheridial cell. This upper cell of the coil may well correspond in some fashion to the conjugating tube or trichogyne of *Pyronema*. The remainder of the coil consisting of several cells serves as the origin for the ascogenous hyphae, being in this respect similar to the ascogonium of the Lichen type. How the fertilization is accomplished in this case, whether there is a migration of a nucleus or nuclei through the apical cell to the cells below as in *Pyronema*, or whether the lower cells serve as auxiliary cells like those in certain red Algae as described by Oltmanns, and as Darbishire thinks is the case in *Physcia*, must be determined by further investigation.

On the whole it is quite certain that we have as yet no final evidence as to the relationships inside the group of the Ascomycetes. In spite of the vast amount of work which has been done, the number of forms which have been fully described in all their stages, even from the standpoint of their external morphology, is surprisingly small as compared with the number which are yet to be worked out. This is due chiefly to the extreme difficulty experienced in finding the earliest stages in the development of the fruits, which at this time are generally buried in the nutrient substratum, and



show no evidence of their existence till they burst through the enveloping medium in an advanced stage of development. When we further consider that the coenocytic condition is very common, and that the function of the cells can only be determined through a knowledge of the behaviour of their nuclei, which are frequently minute in size, it is easy to see why our knowledge has not progressed more rapidly.

In this state of affairs it is certainly scientific as far as possible to withhold judgement as to questions of relationship, and above all to refrain from the attempt to force little-known forms together into artificial groups based on mere superficial resemblances. For the present the question as to the relation of *Pyronema* to the Erysipheae should be regarded as awaiting for its solution a further knowledge of such forms at least as are most closely related to these types, the remainder of the Perisporieae on the one hand and the simpler Discomycetes on the other. It is, however, sufficiently evident that the differences between *Pyronema*, *Sphaerotheca*, and the Lichens, cannot be considered as affording sufficient reason for regarding the Ascomycetes as a polyphyletic group. The ascus with its peculiar method of free cell-formation which has not been observed elsewhere as yet among Algae or Fungi is strong evidence for the morphological unity of the group.

Assuming that the ascocarp is always as it has been shown to be in the cases cited, the asexual spore-fruit, developed from a fertilized or possibly in some cases a parthenogenetic egg and its envelopes, the question as to the nature and relationships of the group of the Exoasci seems a difficult one. It would be very interesting to know, in this connexion, how nearly the method of spore-formation in this group corresponds to the method of free cell-formation as I have found it in the Erysipheae and Discomycetes. I have myself begun some studies looking to this end, which are not yet concluded.

As to the morphology and relationships of the group as a whole it is known that Brefeld regards them as very simple forms in which the asci have not come to be enveloped in more or less closed envelopes. Dangeard (7) describes the

nuclear phenomena in the young ascus as the same in general as in other asci and considers that the asci here as in other cases are oogonia. He finds, however, no such bending of an ascogenous hypha tip and growth of the ascus from its penultimate cell as is seen in the Discomycetes.

De Bary (13, p. 210) puts the group among the doubtful Ascomycetes, and believes they must be regarded as greatly reduced forms, their only relationship with other Ascomycetes being in their hymenia and asci. This latter view seems not improbable, and it seems quite possible to homologize them with certain stages in the development of the Discomycetes. I have already described the relatively independent growth of the ascogenous hyphae in the sub-hymenial layer of such forms as produce relatively large fruit-bodies like *Ascobolus furfuraceus*. In forms with still larger fruit-bodies such as *Peziza Stevensonia*, and in numbers of other *Pezizas* I have examined, this condition is still more striking. The connexion of the sub-hymenial ascogenous hyphae with the ascogonium in *Ascobolus* disappears at quite an early stage. Indeed the ascogenous hyphae seem never to be nourished by material brought up from the mycelium through the ascogonium, but as far as they obtain new food at all to be dependent on the vegetative hyphae among which they grow. They are parasites on the tissues of the mother-plant just as the sporogonium in the Liverworts and Mosses is parasitic on the gametophyte generation. If now these ascogenous hyphae be transferred to some foreign host-plant they might be imagined as continuing their development indefinitely in the tissues of the host, producing crops of asci continuously or intermittently with periods of rest, &c. That is, they might develop quite as does the mycelium of such forms as *Exoascus aureus* on the poplar for example. The mycelium of the Exoasci would, according to this view, correspond to the ascogenous hyphae of the Ascomycetes and not to their ordinary vegetative mycelia. Sexual reproduction might then only occur at long intervals or be entirely suppressed. The ascospores, or the conidia produced from them, might

further come to germinate directly into ascogenous hyphae by a process of apogamy and the suppression of the ordinary mycelium and sexual organs. The condition would then be physiologically analogous to that in those Flowering Plants such as *Allium* and *Funkia*, as described by Strasburger, in which the nucellus-cells, which are potentially spores or spore mother-cells, bud out, push into the embryo-sac, and produce new sporophyte-embryos there in place of the fertilized egg which would normally be developed.

Such suppression of stages in the development of an organism is shown in less degree by the cases of apospory in the Ferns, and the development of new gametophytes from protonemata grown from the vegetative cells of the sporophyte in Mosses, as described by Pringsheim. On the supposition given, the condition in the Exoasci would be quite analogous to what is found in the Rusts according to Raciborski (29), who believes that the original vegetative body in these plants has been almost entirely suppressed, the present mycelium and spore-forms of the group being merely stages intercalated between two phases of the reproductive act, namely, cell-fusion and nuclear-fusion. If the fusions of cells and nuclei in the sexual apparatus, and the fusions of nuclei in the ascus should be found to be two phases of a single sexual act, the suppressed stage in the Exoasci on the view suggested above would be the same as that which Raciborski believes has been suppressed in the Uredineae.

The Exoasci certainly give evidence of considerable specialization in their parasitic habit and in their production of conidia in the ascus, and it seems quite as natural from this standpoint to regard them as a specialized offshoot from the main series of Ascomycetes as to regard them as primitive ancestral forms. The fact that their hymenia are without paraphyses is a minor point which agrees well with the hypothesis I have advanced above, since in the typical Ascomycetes paraphyses are outgrowths of the vegetative mycelia and not of the ascogenous hyphae.

Whether or not this is the true explanation of the reduced



condition of the Exoasci it is at least apparent that the absence of sexual apparatus in them cannot be regarded as evidence of the absence of sexuality in other more typical Ascomycetes. De Bary's view that they are reduced and specialized forms is at least as well founded as the view that they are typical of the primitive Ascomycetes. Dangeard's (7) view also, according to which the Exoasci furnish evidence of the sexual nature of the nuclear fusions in the ascus, cannot be regarded as conclusive, since I have shown that similar nuclear fusions occur in asci arising themselves from a fertilized egg in the Erysipheae and *Pyronema*. Until the method of spore-formation in the group has been worked out the question as to its position and relationships may be left entirely open.

The significance of these nuclear fusions in the ascus is very obscure. Still the entire process in sexual cells and asci is not without analogy elsewhere. Chmielewski (6) has described the nuclear phenomena in the zygospore of *Spirogyra* as consisting of a nuclear fusion at the time of cell-conjugation followed by a double division of the fusion-nucleus, the disappearance of one pair of nuclei so produced, and the fusion of the other pair. Wager (40) has pointed out that this process seems somewhat similar to the series of nuclear phenomena in *Sphaerotheca*. The fertilization occurring in the oogonium would correspond to the first fusion in *Spirogyra*. The period of growth of the ascogonium is then interpolated, followed by a second fusion in the ascus corresponding to the second fusion in the zygospore. In *Pyronema* the same comparison can be made, and further, the disintegration of the two nuclei in *Spirogyra* may be analogous to the cutting off of the two nuclei at least in some cases in the sterile cells at each side of the ascus-bearing cell; but here again the apparent resemblance may have more or less morphological significance.

We certainly need more light on the phenomena of chromosome-reduction, and the method and time of its occurrence in the lower plants, before a conclusion can be reached on this

point. The number of chromosomes must be first determined in both vegetative and reproductive cells at all stages. As a contribution in this line I have attempted to count the visible number of chromosomes on the spindles in a large series of nuclei in the divisions in the ascogenous hyphae just prior to ascus-formation, and also in the asci themselves. The figures are so small and the chromosomes so crowded that it is extremely difficult to get satisfactory results. The chromosomes overlap and are bunched in many cases so that neither polar nor side views reveal the exact number with certainty. The larger nuclei in the first division in the ascus are by far the most favourable for this work, and I am of the opinion from a study of a large series of cases that there are ten chromosomes present at this stage. In *Peziza Stevensoniana* I was able to determine that their number is not changed in the three divisions in the ascus, and I am of the opinion that the same is true for *Pyronema*, though the smaller size of the nuclei in the second and third divisions here makes them much less favourable objects of study.

As to the number of chromosomes shown in the division in the ascogenous hyphae I am uncertain. As is seen from Figs. 25-28 the visible number of chromatin granules on the spindle is variable. This is doubtless due to massing of the individual chromosomes in some cases and to the fact that they may lie vertically above one another in the spindle. It is also difficult to determine in many cases whether the separation of the daughter-chromosomes in the equatorial plate has taken place. Polar views do not seem much more favourable for determining the number than do side views of the spindle as will be seen from the upper nuclei in Figs. 27 and 28. All the Figs. 25-28 have been drawn with great care to show exactly the number of granules visible in each case. I am inclined to the view that the same number, that is ten, is present here as in the divisions in the ascus described above. Whether this is the double number which results from the fusion in the oogonium, or whether reduction has already taken place is uncertain. I have already pointed

out the possibility that reduction may occur in the ascus, the threefold division of the ascus-nucleus being possibly homologous with the double divisions of the spore mother-cells in the higher plants ; but the simultaneous divisions just prior to the formation of the ascus with the production, in some cases at least, of two nuclei which develop no further, suggest the possibility that reduction may occur at this stage. It is quite possible that the stages in development here are not to be homologized with those in the higher plants where a definite alternation of generations occurs ; still the ascocarp certainly plays the same rôle physiologically as the sporophyte of the higher plants, and the morphological equivalence of the structures concerned is at least strongly suggested. From this standpoint reduction might occur at either of the stages mentioned above. Further study of the chromosome number in various forms among the thallophytes is necessary before a definite conclusion can be reached on this point.

MADISON, WISCONSIN, *May 22, 1900.*



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## EXPLANATION OF FIGURES IN PLATES XIX-XXI.

Illustrating Professor Harper's paper on *Pyronema confluens*.

### PLATE XIX.

All figures were drawn with the aid of the *camera lucida*, and with the Zeiss apochromatic objectives. Figs. 3-10, 12-15, 17 *a*, 17 *b*, 19-22, and 24 with obj. 2 mm. compens. oc. 4. Fig. 11 with obj. 2 mm. oc. 6. Figs. 2, 16, 16 *a*, 16 *b*, 31, 42, and 43 with obj. 2 mm. oc. 8. Figs. 25-30, 32-41 with obj. 2 mm. oc. 12. Fig. 18 with obj. 8 mm. oc. 12. Fig. 23 with obj. 8 mm. oc. 4.

Fig. 1. Rosette of three pairs of sexual cells drawn from surface view, the group slightly flattened by pressure.

Fig. 2. Branched mycelial cell showing nuclei and granules on the septa.

Fig. 3. Young pair of sexual organs with vegetative cells below. Trichogyne appears as a mere papilla on the oogonium.

Fig. 4. Older pair, trichogyne not yet cut off from oogonium. Antheridium cut transversely.

Fig. 5. Longitudinal section of antheridium. Trichogyne cut obliquely showing smaller nuclei.

Fig. 6. Oogonium and trichogyne cut longitudinally. Oogonium stalk with budding vegetative hyphae. Trichogyne with hyaline beak, its nuclei swollen and transparent.

Fig. 7. Longitudinal section of antheridium; oblique section of trichogyne showing the fusion-pore. Nuclei of antheridium not yet disintegrated as they commonly are at this stage.

Fig 8 *b*. Oblique tangential section of antheridium. Trichogyne cut transversely near its base and apex. Pore forming by solution of walls between trichogyne and antheridium. *a*. Remainder of same trichogyne from next section showing disintegrated nuclei.

Fig. 9. Oblique section of antheridium and trichogyne showing receptive spots of each and formation of pore.

Fig. 10. Pore complete and fragments of dissolved wall lying near in the trichogyne. Hypothecal hyphae springing from stalk-cells. Nuclei in trichogyne disintegrated.

Figs. 11 and 12. Slightly later stage, receptive spots becoming spongy and vacuolar.

Fig. 13. Nuclei beginning to migrate from antheridium to trichogyne. Stalk cut obliquely not showing hypothecal hyphae.

Fig. 14 (Plate XX). Trichogyne filled with nuclei from the antheridium. Nuclei collecting in oogonium. Antheridium curved around the trichogyne so that the latter appears in section to cut through it.

Fig. 15. Basal wall of trichogyne dissolved and male and female nuclei collected in a dense mass in centre of oogonium and fusing in pairs. Nuclei still present in trichogyne and upper end of antheridium. Ascogenous hyphae appearing as buds on oogonium.

Fig. 15 *a*. Another oogonium at about the same stage as the last.



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Figs. 16, 16*a*, and 16*b*. Parts of tangential sections of the central mass of nuclei in different oogonia showing groups of fusing nuclei in various stages. 16*a* slightly less magnified.

PLATE XX.

Figs. 17*a* and 17*b*. Two sections from the same oogonium showing the separation of the nuclei after fertilization. Young ascogenous hyphae containing nuclei. Basal wall of trichogyne again present.

Fig. 18. Approximately horizontal section through a rosette of sexual cells showing distribution of vegetative branches which are to form hypothecium. One of the antheridia does not appear in this section.

Fig. 19. Section of older oogonium showing the distribution of the ascogenous hyphae among the young hyphae of the hypothecium.

Fig. 20. Section of an ascocarp showing, approximately, the hymenium and hypothecium developed from a single pair of sexual cells. *a*. Portion of ascogenous hypha containing unfertilized supernumerary nuclei.

Fig. 21. Old oogonium with branching trichogyne, which has united with two separate antheridia.

Fig. 22. Oogonium older than that in Fig. 20 and showing large fertilized and smaller unfertilized supernumerary nuclei.

Fig. 23. Semi-diagrammatic drawing of a section of an ascocarp in which the first asci are ripening.

Fig. 24. Storage cells from the hypothecium out of which paraphyses are developed.

Figs. 25, 26. Tips of ascogenous hyphae, from the hymenium, showing pairs of nuclei in division.

PLATE XXI.

Figs. 27-29. Tips of ascogenous hyphae showing pairs of nuclei in division.

Fig. 30. Tip of ascogenous hypha showing four nuclei formed by the division of the two nuclei shown in preceding figures.

Fig. 31. Later stage than that in Fig. 30. Dome-shaped young ascus containing two nuclei. A second ascus forming below.

Fig. 32. Slightly older ascus showing two nuclei and two supporting cells.

Fig. 32*a*. Same stage showing a single supporting cell.

Fig. 33. Nuclei in young ascus fusing.

Fig. 34. Young ascus with single nucleus formed by fusion of the two nuclei shown in preceding stage.

Fig. 35. Primary ascus nucleus in division.

Fig. 36. Two young daughter-nuclei formed by division of primary ascus nucleus.

Fig. 37. Division of the secondary ascus nuclei.

Fig. 38. Dispirem stage of same division.

Fig. 39. Four-nucleated stage in development of ascus.

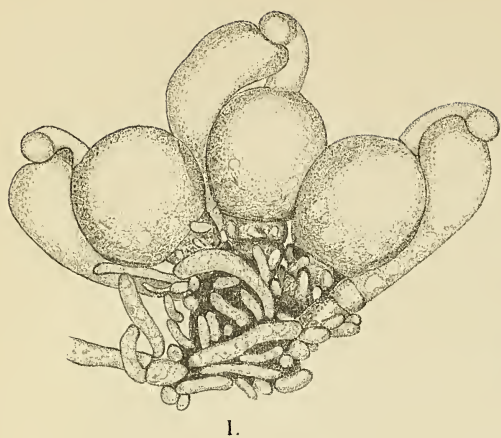
Fig. 40. Eight-nucleated stage. Nuclei with beaks and asters at the tips of the beaks.

Fig. 41. Stage in the cutting out of the ascospores by the folding back and fusion of the rays of the aster.

Fig. 42. Young ascospores enclosed only by plasma-membranes.

Fig. 43. Nearly ripe ascospores with fungus-cellulose wall.





1.



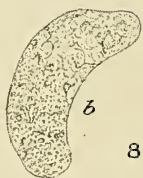
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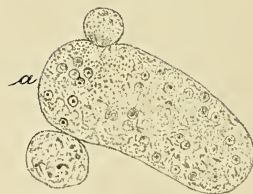


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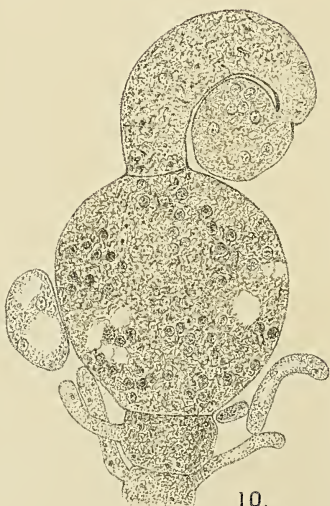
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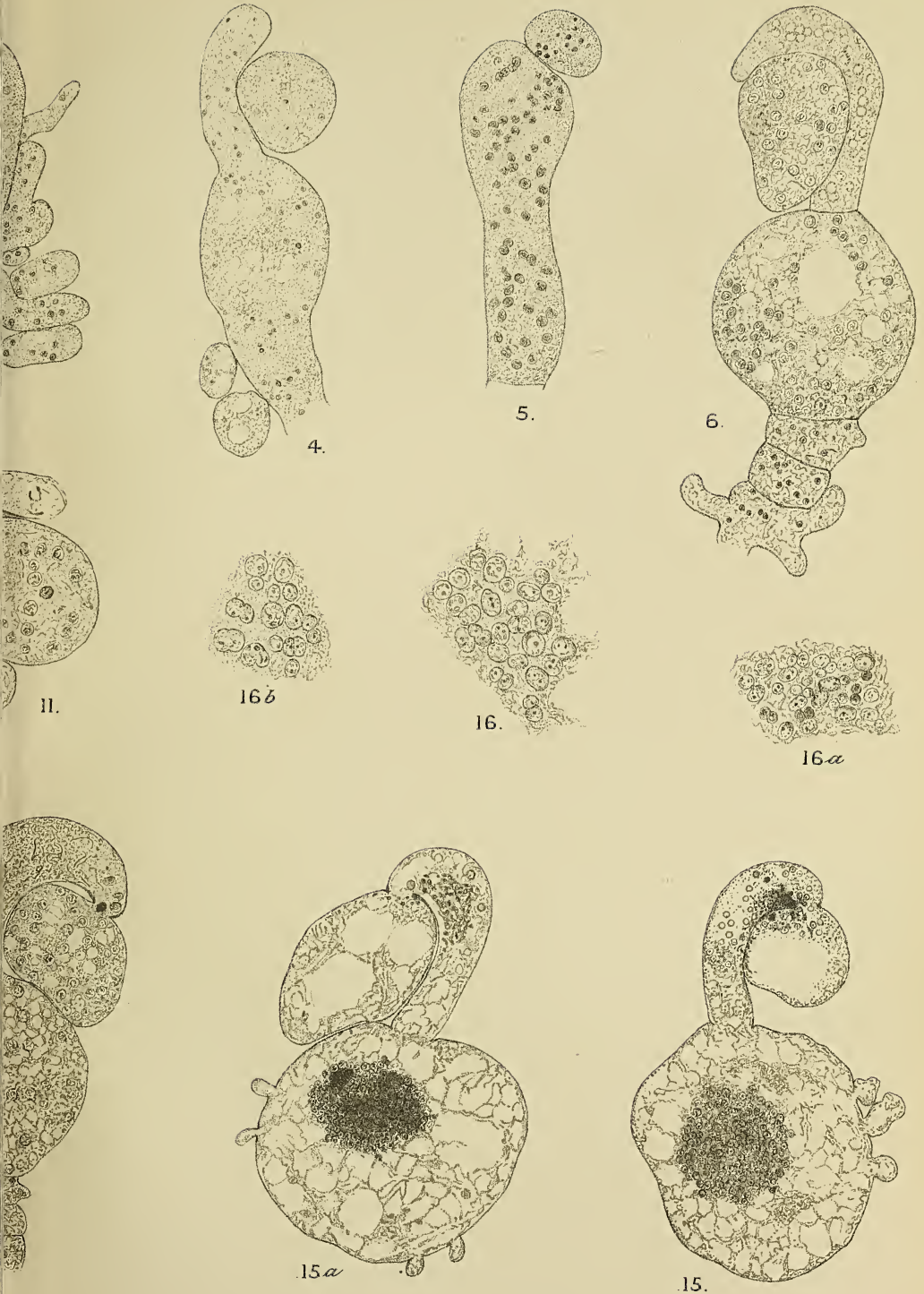


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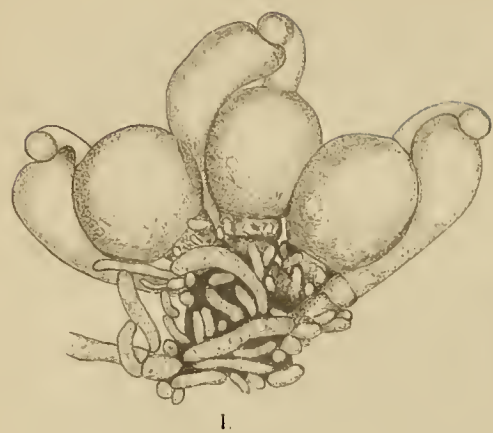


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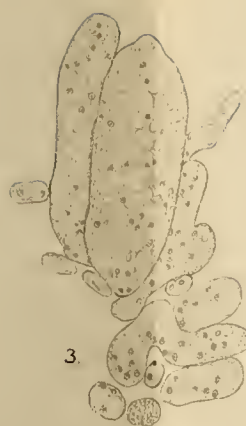




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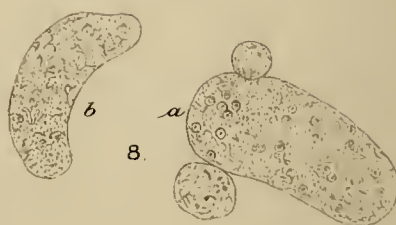
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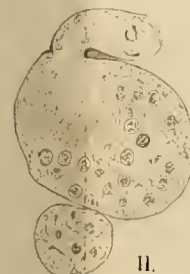
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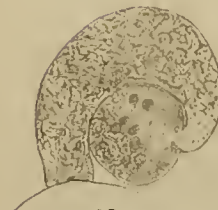
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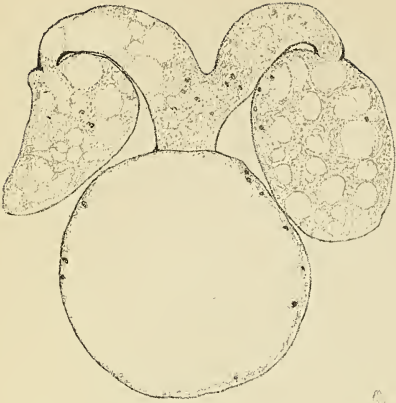
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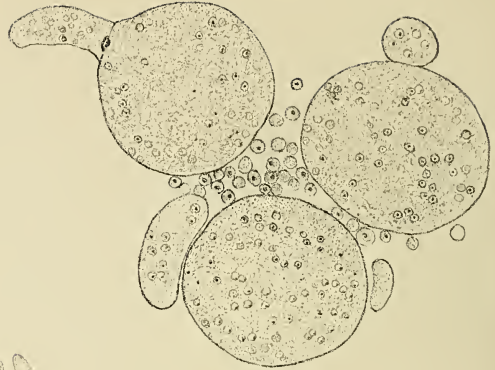








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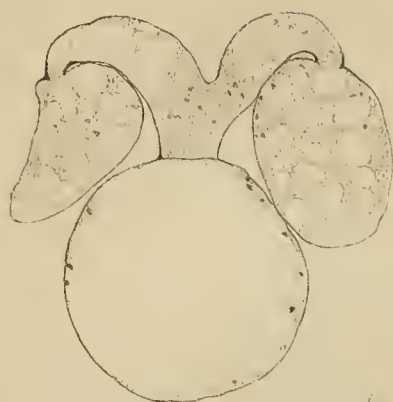


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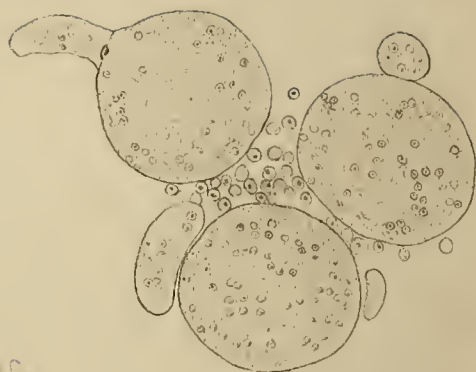


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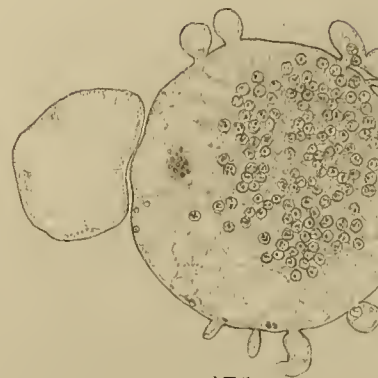
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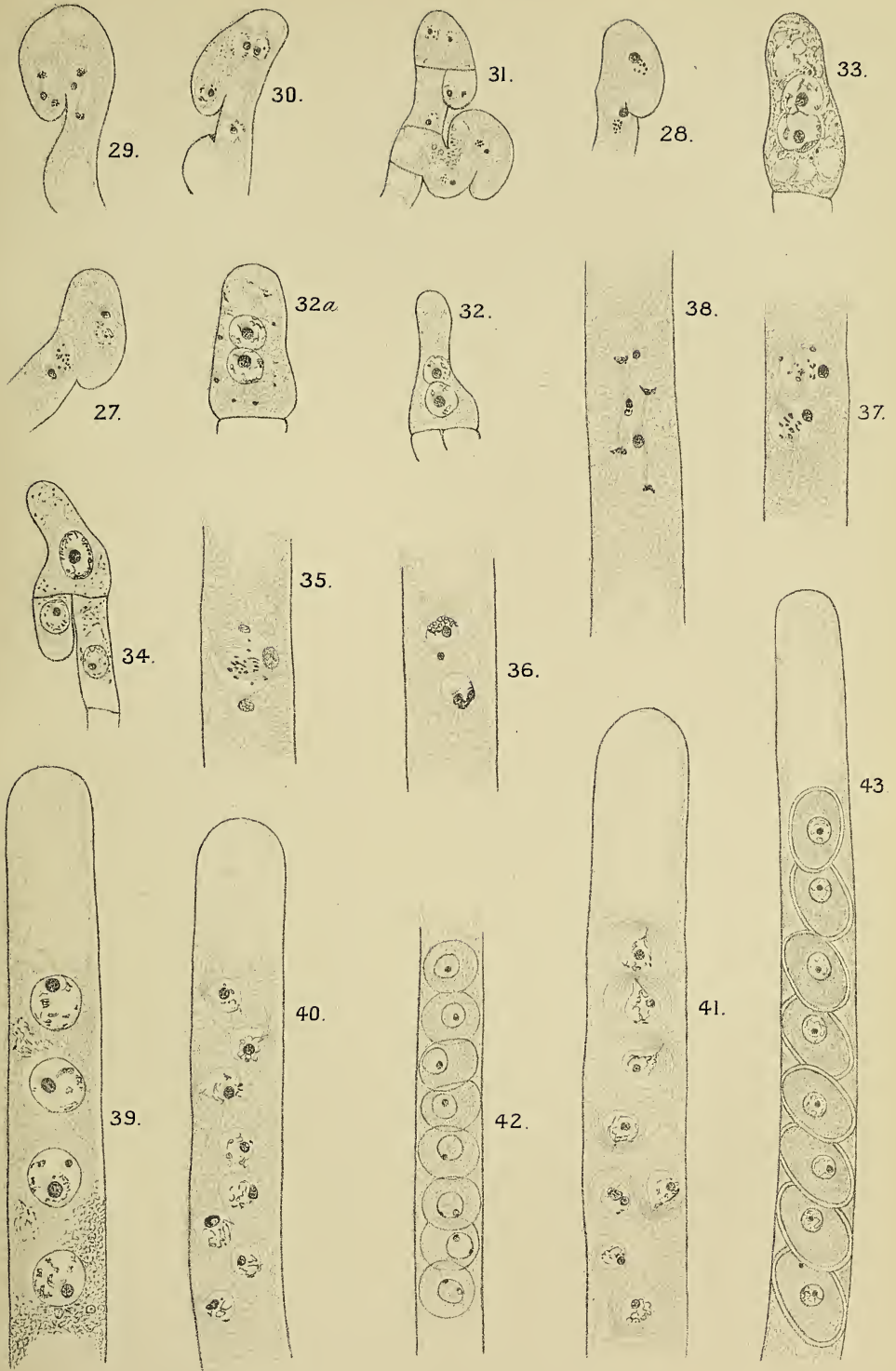
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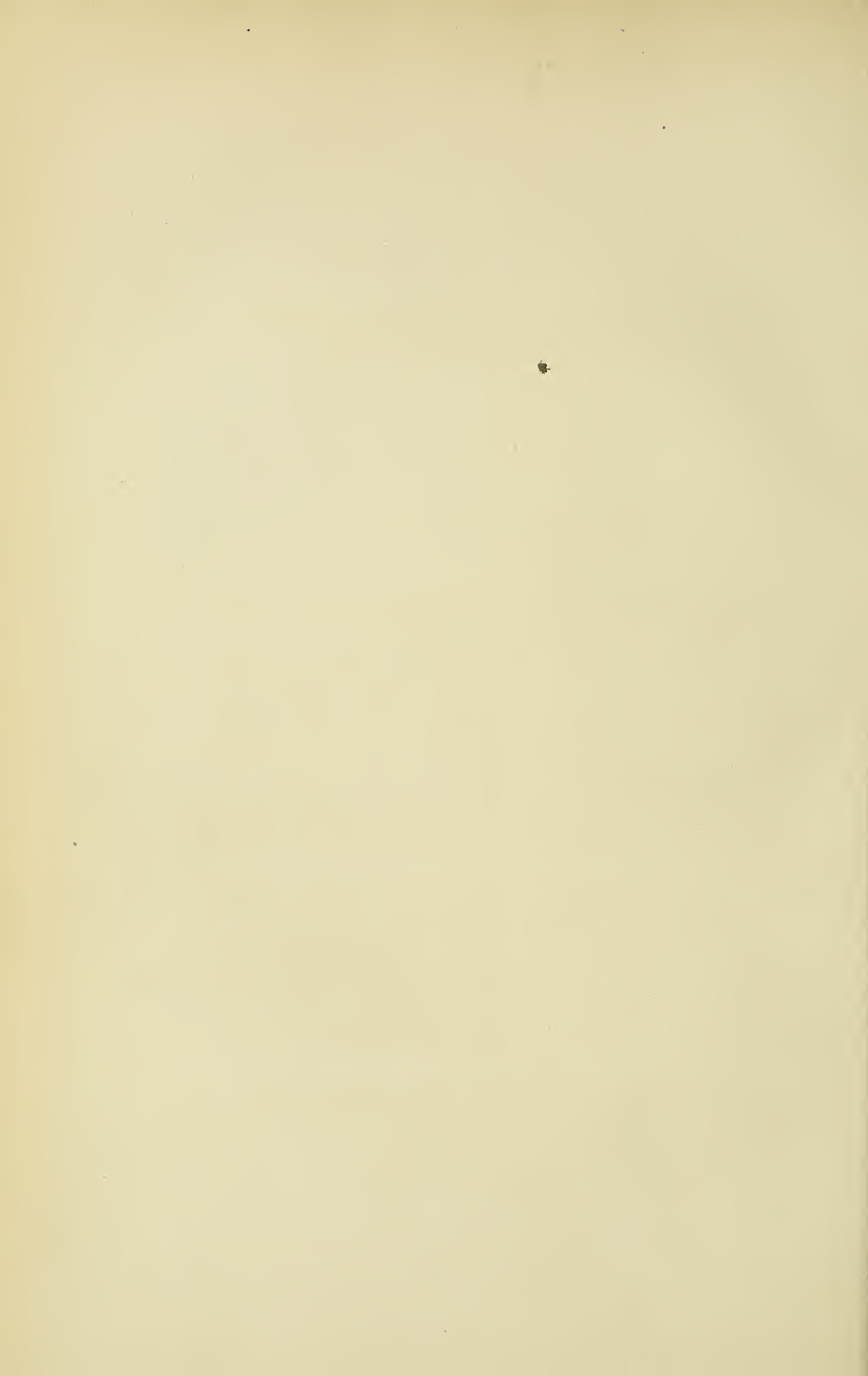


24.











# A Short Memoir of Ito Keisuké, Rigaku Hakushi (Doctor of Philosophy)<sup>1</sup>.

BY

TOKUTARO ITO, D.Sc.



With a Portrait (Plate XXII).



THE following translation of M. Tokutaro Ito's valuable memoir of his celebrated grandfather is for the most part a full one, but occasionally omission and compression have been exercised. It is hoped that it will be found interesting, as the memoir of a Japanese botanist of Old as well as of New Japan, who was also one of the principal pioneers of western science in Japan, written by a Japanese for Japanese readers.

M. Tokutaro Ito's memoir was originally published in the *Toyo Gakugei Zasshi* (Journal of Oriental Science and Art) in 1898.

I have to thank my friend Mr. Kumagusu Minakata for help in reading some of the proper names and in other ways.

The rendering of Japanese titles, many of which are new since I left Japan twenty years ago, has given me considerable trouble, and I am not sure that I have always hit upon the exact English equivalents—perhaps because they do not exist.

F. V. D.

<sup>1</sup> The editors are much indebted to Mr. F. Victor Dickins for translating the original Japanese memoir, and to Dr. Tokutaro Ito for readily consenting to its publication.

The venerable Doctor's name is Keisuké. He is known also as Kinkwa, and again as Seimin<sup>1</sup>. Lastly he calls himself Taikosan Sho (a faggot of Mt. Taiko) after a country residence he possesses of that name in the village of Ueno, in the district of Aichi, in the province of Owari<sup>2</sup>.

He was born on the 27th of New Year's month<sup>3</sup> of the 3rd Kyowa<sup>4</sup> (A.D. 1803) in Gofuku<sup>5</sup> Street in Nagoya in Owari. His father, Nishiyama Gento, followed the profession of medicine. His mother's name was Noma. There were three sons and one daughter of the marriage. The eldest son was called Sonshin, the second son was the venerable doctor. Sonshin entering the family of Okochi, Keisuké became the heir. In his youth he was called Nishiyama Sachu, but in accordance with his father's wish the name was discarded and the old family name of Ito was resumed. Following in the footsteps of his father and elder brother, he adopted the profession of medicine. From boyhood he was fond of collecting plants and inquiring their Chinese and Japanese names from his father and brother.

In Bunkwa and Bunsei<sup>6</sup>, in company with Mizutani Sukeroku he made a tour through the provinces of Owari, Mikawa, Isé, Shima, Mino, and Shinano, collecting plants, animals, and minerals.

In 1821, being nineteen years old, he went to Kyoto and made the acquaintance of many well-known botanists. With

<sup>1</sup> In Old Japan most writers, artists, &c. assumed various literary or artistic names at different periods of their life. The family name came first always—thus Ito Keisuké, now commonly written Keisuké Ito.

<sup>2</sup> The Japanese names of provinces are given. The Japanese, after an apparently arbitrary fashion, call some provinces by their native, others by their Chinese, names. Thus Owari is more often known as Bishu.

<sup>3</sup> I make this date, according to the old calendar, to be Feb. 20, 1803.

<sup>4</sup> A *nengo* or year-period of Old Japan. These had more or less fanciful Chinese names. Kyowa might mean Enjoyment of Peace. In 1867 the *nengo* was named Meiji (Illustrious Rule) and is to be conterminous with the reign of the present Emperor.

<sup>5</sup> Gofuku means 'clothes.' It is a Chinese word, the literal signification of which is 'Go (Wu) clothing,' indicative of Wu as one of the sources whence Chinese civilization was introduced into Japan.

<sup>6</sup> Bunkwa, 1804-17. Bunsei, 1818-29.

the help of the venerable Fujibayashi Taisuké he began the study of foreign learning. From time to time he botanized in the hill-tracts of Hiyei, Kibuné, Kurama, and Atago, and in the following years in the provinces of Yamashiro, Settsu, Yamato, Isé, Shima, Mikawa, Totomi, and Suruga. He was then invited to Yedo, and enjoyed the hospitality of the venerable Udagawa Yoan<sup>1</sup>, with whom he spent a month collecting in Nikko, whence he returned to his native Nagoya by Haruna and Myogi in Kozuke, and Kiso in Shinano. A little earlier the German botanist Ph. Fr. von Siebold had arrived in Japan and taken up his residence at Nagasaki. In 1826, desiring to behold the Shogun's court, he went up to Yedo, and on the way, at Atsuta, a coolie-relay station in Owari, met Mizutani Sukeroku and Okochi Sonshin, together with the venerable subject of this memoir—to the great pleasure and profit of all, as one may well believe. The meeting is mentioned in Siebold's *Nippon*:—‘Ich lernte hier die meinen Untersuchungen später so nützlich gewordenen Ito Keiské und Okutsi Sonsin kennen’ (Siebold: *Nippon*, vol. i, Abteilung I, p. 168). Ito Keisuké could scarcely bear to part from Herr von Siebold, and accompanied him as far as Narumi. When they separated, von Siebold expressed his great desire to see his fellow traveller again at Nagasaki, and thenceforth Ito Keisuké could not rest until he had gained permission from his father and elder brother to make the journey to Nagasaki. It was with no little delight he set out on the journey, and in his old age he often recalled the pleasure he felt, and frequently spoke to the present writer of the delightful anticipations of that time. In 1827, in the 9th month, being in his 25th year, he arrived at Nagasaki, and lodged in the house of the Chief Interpreter Yoshio Gonnosuké. He lost no time in calling upon von Siebold, who was delighted to see him again. The whole of the time

<sup>1</sup> Udagawa Yoan was a very remarkable man, who took a large part in introducing western science to his countrymen. He published a work on *seimi* (*chemie*) in 1837 (*Seimi Keiso*), and earlier still, in 1834, an elementary treatise on western botany, *Shoku-gaku Keigen*.



spent at Nagasaki, from the first day to the last, in the spring of the following year, was occupied in botanical work with the German botanist. We can well picture to ourselves how delightful he must have found such an intercourse. At this time, von Siebold occupied a house in a part of Deshima known as Hanabatake (the Garden Ground), about one *chō* (60 ft.) square filled with plants native and foreign, grown for examination. The beds were arranged by von Siebold himself in rows symmetrically placed opposite each other, and must have presented a very agreeable appearance. The house was an ordinary upper-storied Japanese dwelling. The venerable doctor went there daily to prosecute his studies. At this time there was a gate at the entrance to Deshima occupied by a guard (*saguriban*—examining watch) consisting of several yakunin<sup>1</sup>, who searched the folds and sleeves of every person who passed in or out. Ito was allowed to carry his herbaria and packets of plants in and out, but these had to be searched each time; in fact scientific investigations were then carried on under many difficulties scarcely realizable by men of science at the present day. Among Ito Keisuké's fellow students at Nagasaki were Kō Ryōsai, Takano Choyei<sup>2</sup>, Oka Kennosuké, Kaku Saichiro, and Hayashi Dokai.

After a stay of some six months Keisuké was recalled home by family affairs. Herr von Siebold was sorry to lose him, and gave him as a farewell gift a treasured copy of Thunberg's *Flora Japonica*, bidding him use it diligently as a help to his studies. His pupil passed through Kiushu and Banshu, collecting plants on the way, and on reaching Nagoya resumed the practice of medicine, but whenever he could find time read Thunberg diligently and made it the foundation of his further studies, in the first place collating the Latin names with the Chinese and Japanese names of native plants, so as to adjust and complete their nomenclature.

With the aid of Thunberg's work, and in the light of the

<sup>1</sup> Rather a European than an ordinary Japanese designation of a Bakufu official.

<sup>2</sup> Takano Choyei was a man of considerable ability. He was among the first to recognize the value of the English alliance. He was prosecuted and finally committed suicide. He was posthumously raised to the fourth class last year.—K.M.

*Systema Naturae* of Linnaeus, he prepared his *Taisei Honzo Meiso*<sup>1</sup> in 3 vols., which he presented to his Daimyo in 1829. Herr Nordenskiöld, in his *Voyage of the Vega*, referring to Thunberg's *Flora*, gives a portrait of the venerable doctor [as he then appeared?]. On the covers of this work, with the object of correcting a popular error, Keisuké represented a *yamabuki* fruit (*Kerria Japonica*) and an *ichijiku* flower (*Ficus*) accompanied by a line from an old poem—

‘*Mi no hitotsu dani naki zo kanashiki!*’

*Not so much as a single fruit to be seen, alas!*<sup>2</sup>

With the desire of introducing the Linnean system into Japan, Udagawa Yoan had some time before explained it in a book called *Botanika kyo*, published in 1822, but unfortunately the work was little noticed. Our venerable doctor much regretted this neglect, and in 1879 desired me to reprint it together with the *Seisetsu kuwansho kyo* of Yoshio Shunzo published also in 1822. When Herr von Siebold returned home he took with him over ten portfolios of dried plants given him by Ito Keisuké, which are now preserved in the Leiden Museum. Professor Geerts refers to this collection in the following words:—

‘Mr. Ito Keiské, le célèbre botaniste Japonais, qui a le premier observé et décrit une quantité de plantes nouvelles, et enrichi le musée de Leyde d’un herbier fort intéressant et très-précieux. Il a publié en 1823 une traduction critique de la Flore Japonaise de *Thunberg*, comprenant trois volumes in-8°. Ce livre intitulé *Tai-sei-hon-zo-mei-su* est très-difficile à trouver aujourd’hui chez les libraires de Kiyoto ou de Yédo’ (Geerts: *Les Produits de la Nature*, vol. i, Introduction, 1876).

<sup>1</sup> A list of Ito Keisuké's works is appended to the memoir.

<sup>2</sup> The Stanza is in the *Kokinshu* (Poems Old and New; 10th Century):—

<i>Nanaye yayé</i>	The seven-petalled, the eight-petalled
<i>hana wa sakedomo</i>	Flower! though it bloom
<i>Yamabuki no</i>	on the Yamabuki, yet never a fruit,
<i>mi no hitotsu dani</i>	not so much as a single fruit
<i>naki zo kanashiki.</i>	doth it show, alas the Yamabuki!

The Japanese notion (derived from China) was that *Kerria* had no fruit and *Ficus* no flower.

Professors Hoffmann, Miquel, and Schultes examined these portfolios, and Professor Miquel gave an exhaustive description of the *Herbarium Botanici versatissimi*, Ito Keisuké, *in prov. Owari ins. Nippon degentis, Sieboldi amici*, forma octava maxima XIII volumina (quaedam alia deperdita).

On his return to Nagoya from Nagasaki Ito Keisuké began to practise medicine on the Dutch system. At this time the Chinese system was in vogue, and the innovation was looked at askance, in fact was suspected of sorcery, and the physician incurred some odium.

Disregarding this danger, Ito persisted in his efforts to promulgate Dutch learning and science, now explaining the grammar and collecting vocabularies of Dutch, now insisting upon the advantages of vaccination, or setting forth the principles of chemical science and the progress of western philosophy. Meanwhile he prosecuted the new system of medicine until at last pupils began to gather around him and patients to besiege his doors. For forty years he continued to practise foreign medicine at Nagoya, but in the intervals of leisure afforded during a busy career, showed a sustained interest in natural science. In 1832 and 1838 he botanized in Shinano, in 1852 and 1855 in Omi, Yamashiro, Settsu, Isé, and Shima, in conjunction at various times with Yoshida Heikuro and Iinuma Yokusai<sup>1</sup>.

In 1827 he established a Museum of Pharmacy in Nagoya, and later in 1858 a Physic Garden in the same town and a Natural History Museum.

In 1837 a famine raged in Japan. Moved by the pitiable condition of the distressed people, our venerable doctor wrote a pamphlet (*Kiuko Shokubutsu Benran*) on Edible Plants which the Daimyo, struck by its utility, ordered to be printed, and caused thousands of copies to be circulated throughout his fief in the provinces of Owari, Mikawa, and Mino. The next year a great fire occurred in Yedo, and the Nishimaru quarter of the Castle (seat of the Bakufu Government) was burnt down. To procure *hinoki* (*Chamaecyparis*) timber for rebuilding the

<sup>1</sup> Author of the well-known *Somoku Zusetsu* (*Illustrated Flora*).



castle, a government commissioner was associated with an officer of the *han* (fief), and hundreds of coolies were collected to explore the forests of Mt. Kiso, of which opportunity Ito availed himself to study the flora of the district, accompanying the party as medical officer. For several months he lived in a hut and diligently searched hill and valley for plants, finding many rare and interesting specimens.

Ito, as already mentioned, had previously given much attention to western learning, and explained the essentials of foreign languages. In 1841 he published his *Yojihen* for the use of beginners, as a result of which very many persons desirous of acquiring foreign languages sought his aid. In 1847 he was made a Chief Superintendent to the Daimyo (*Goyōnin shihai*), and the year after was much occupied with editing and translating important foreign works. About this time he revised the *Nagara-gawa Kijishi* and arranged the *Hyochu Shisho*. He also translated Salmon's *Nihon hen* and wrote a treatise on foreign saltpetre, founded on a Dutch work.

In 1852 he was appointed a Vaccination Inspector, and began his duties with the establishment of a station at Nagoya.

At this juncture rumours were rife of the coming of a foreign squadron, and great was the confusion and consternation they caused. Ito ordered three hundred cannon to be cast and presented them to the Daimyo, receiving a large sum of money by way of reward.

In 1854, when the foreign ships appeared, he was appointed a secretary, and afterwards furnished much information on foreign affairs.

In 1858 he reprinted the *Oranda Chiri Shoho* (Geographisch Zakboekje), upon which he afterwards founded his *Yochi kiryaku* (Short Account of our Globe) for the use of beginners.

In 1859 he was nominated a medical adviser, and a teacher of the art of translation from foreign languages. The next year he was made a member of the *Banshochosho* (office for examining barbarian writings), and called to Yedo to take part in the duties of the department of Products and Manu-

factures. In the next year he was rewarded with five pieces of silver on account of his diligence in promulgating vaccination. In 1863 von Siebold returned to Japan and settled at Yokohama, where the Bakufu sent Ito Keisuké to report upon the subject of natural science. Herr von Siebold was delighted to see his old friend again. The same year Ito Keisuké was obliged, through ill-health, to resign his office in the *Ban-shochoshô*, and returned to his native town. At this time cholera (*bôsha*) was ravaging the country, and he issued a small handbook of precautionary measures which was widely circulated. In 1865 the Daimyo appointed him his family physician. After the Restoration, in 3rd Meiji (1870), he was nominated an official by the new Government, and was summoned to the capital to be created a member of the University, with the degree of *shô hakushi* [sort of *licencié ès-sciences*].

In 1871 he was given a professorial appointment under the Ministry of Education. He afterwards became Deputy Assistant Compiler (*henshu gonnosuké*), and in 1872 received 7th class rank, being specially employed in the section of Natural Science. He was appointed Compiler (*Henshukwa*) in 1873. He was now busy with his *Nippon Sambushû*, of which he officially published six parts, dealing with the provinces of Yamashiro, Musashi, and Omi. In 1874 he gave to the world the first part of the Herbs section of the *Nippon Shokubutsu Zûsetsu*—Illustrated Japanese Flora—the preparation of which had occupied him during many years. His son, who had previously written on Pharmacy and Elementary Botany, compiled the index. Professor Geerts thus refers to the work:—‘Mais il nous faut surtout parler du dernier ouvrage que vient de publier, malgré son grand âge, Mr. Ito Keiské, en collaboration cette fois avec son fils, Mr. Ito Udzuru. C’est le *Ni-honshoku-butsu-dzûye*, ou *Description des plantes Japonaises encore inconnues*. Dans le premier volume de cet ouvrage, publié en 1874, Mr. Ito Keiské a décrit et dessiné environ une cinquantaine de plantes nouvelles découvertes par lui. Ces plantes n’avaient été déterminées d’une manière

aussi exacte dans aucun ouvrage Japonais. Mr. le Docteur Savatier a écrit une préface pour ce livre, et il y fait à juste titre un grand éloge du zèle et du savoir du doyen des botanistes Japonais contemporains. Dans ce dernier travail de Mr. Ito Keiské, le nom scientifique figure en caractères romains à la suite de la plupart des plantes qui y sont décrites. Espérons que le vénérable savant vivra encore assez pour continuer et terminer cet ouvrage si intéressant et si utile' (Geerts, *loc. cit.* p. 37).

In 1877 he was made extraordinary Professor of Philosophy (science) in the Tokyo University, and appointed to a special post in connexion with the Botanical Gardens. In addition he undertook duty in relation to the Educational Museum.

The same year he completed the first part of the *Koishikawa Shokubutsu-en Somoku Mokuroku*, and further instalments of the *Nippon Sambusshi*.

In 1880 he became a Director of the Koishikawa Gardens, and published the second part of the *Mokuroku*. The next year he was made a Professor in the University. In collaboration with Kaku Hika, the brother of Kaku Saichiro, he published the first volume of the *Koishikawa Shokubutsu-en Somoku Zusetsu*, and in the following year the second volume appeared. In 1879 he had been elected a Fellow of the *Tokyo Gakushi Kwai-in* (Tokyo Academy of Learning). He afterwards published in the Journal of the Society an essay on the 'Rise and Course of Natural Science in Japan,' and a paper called *Kwashi zakki* (Botanical Notes). Other articles of his may be found in the Transactions of the Yōyosha (Society for promoting Culture, founded about 1872 by the well-known publicist, Fukuzawa and others).

His 'Edible Plants' and 'Poisonous Plants' were mentioned in the *Kwampo* (Official Gazette). In 1880 he received a silver medal from the Royal Academy of Stockholm, in 1881 a second class medal from the International Geographical Congress at Venice, and in the same year he was elected a corresponding member of the N. China Branch of the Royal Asiatic Society.



In 1877 and 1881 he was appointed an Inspector of the Exhibition of National Industries. In 1887 he was made a member of the 4th class of the Order of Merit. In 1888 the degree of Doctor of Philosophy (*Rigaku Hakushi*) was conferred upon him, and in 1893 he was raised to the lower fourth official rank (*Fushii*).

The venerable botanist has discovered many new Japanese plants—to not a few of which his name has been given, as to the *shimobashira* called *Keiskea* by Prof. Miquel.

He has now attained his ninety-sixth year, and is still hale and hearty. His interest in systematic botany is undiminished, and he shows no sign of flagging powers. He still has by him hundreds of unpublished papers on botanical subjects, by-products of the labours of a long life.

Translated by F. VICTOR DICKINS.

May 17, 1900.

Ito Keisuké, I rejoice to say, is still alive in his ninety-eighth year. He has lately been selected by his countrymen as one of 'the Twelve Heroes of Modern Japan.'—F. V. D.

A LIST OF ITO KEISUKÉ'S PRINCIPAL WORKS.

1. TAISEI HONZO MEISO : Western Botanical Nomenclature, 3 vols., 1829.
2. KIUKO SHOKUBUTSU BENRAN : Handy Book of Edible Plants, 1837.
3. YOJI HEN : Book of Foreign Characters, 1 vol., 1841.
4. NAGARAGAWA KIJISHI : Poems in praise of the River Nagara, 2 vols., 1848.
5. HYOCHU SHISHO : A Collection of Loyal Poems, 3 vols., 1850.
6. SHOSEKI HEN : Treatise on Saltpetre, 3 vols., 1854.
7. BOSHABYO TE-ATÈ : Handbook of Precautionary Measures against Cholera, 1 vol., 1863.
8. NIPPON (or NIHON) SAMBUSHI : Products of Japan, 11 vols., 1873-1877.
9. KOISHIKAWA SHOKUBUTSU-EN SOMOKU MOKUROKU : Catalogue of Plants in Koishikawa Gardens, 2 vols., 1877-1880.
10. KOISHIKAWA SHOKUBUTSU-EN SOMOKU ZUSETSU : Illustrated Description of Plants in Koishikawa Gardens, 2 vols., 1881-1884.
11. KIUKO SHOKUBUTSU SHUSETSU : Edible Plants (Government Edition), 1884.
12. YODOKU SHOKUBUTSU, SHUSETSU : Poisonous Plants (Government Edition), 1884.
13. KINKWA KOGWA FU : An account of ancient tiles (an antiquarian work by Kinkwa, one of the names of Ito Keisuké), in the 16th volume of the Journal of the Tokyo Academy of Science, 1894.
14. SALMON'S 'Nihon hen' : Perhaps this is No. 395 of Pagès, Bibl. Jap., 'Salmon, Th., Tegenw. staat der keizerrijken China en Japan, uit het Eng. ver., Amst. 1729, met pl. en kaarten.'







ITŌ KEISUKÉ, KNOWN AS THE VENERABLE KINKWA  
AGE 96 (IN 1898)



# The Structure of *Isoetes Hystrix*.

BY

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*Honorary Keeper of the Jodrell Laboratory, Royal Gardens, Kew,*

AND

T. G. HILL,

*Late Marshall Scholar, Royal College of Science, London.*



With Plates XXIII and XXIV, and two Figures in the Text.



## INTRODUCTION.

IN April and May, 1899, one of us received, through the kindness of Mr. E. D. Marquand, a number of fresh specimens of *Isoetes Hystrix*, collected by Mr. George Derrick, a botanist resident in Guernsey, at the well-known locality on l'Ancrese Common, in that island, the only known British habitat for the species<sup>1</sup>. To both the gentlemen mentioned our best thanks are due. This material, which included plants of very different ages, afforded a welcome opportunity for a re-investigation of the structure in this form, which is of considerable interest as being one of the terrestrial species,

<sup>1</sup> See Mr. Marquand's note on the genus *Isoetes*, in Trans. Guernsey Soc. of Nat. Science, 1889, p. 123.



of which, according to Baker's arrangement, there are only two (*I. Hystrix* and *I. Duriaei*) among the forty-nine species described in his 'Fern-Allies.' Although so much work has already been done on *Isoetes*, such a re-investigation does not appear superfluous, in view of the widely different views which prevail as to the affinities of the genus, and of the ever-increasing interest which attaches to the surviving Pteridophyta, as we become better acquainted with their history in past ages.

Our own observations relate entirely to the structure and development of the vegetative organs, for it is these points which appear most to need further elucidation, while the reproductive and embryological phenomena have been thoroughly investigated by our predecessors.

A terrestrial species, such as *I. Hystrix*, is clearly the most favourable for anatomical investigation, as the tissues are more characteristically developed than in the reduced, aquatic forms. In a genus, such as *Isoetes*, however, with so great a preponderance of aquatic or sub-aquatic representatives, we must regard it as an open question whether these, or the exceptional terrestrial forms, are the more primitive. In any case there can be no doubt that the terrestrial species share, to a considerable extent, in the reduction which characterizes the genus as a whole. (See below, p. 443.)

As regards the plan of the paper, after a few general descriptive remarks, the structure of the stem will be considered, then that of the leaves, and finally that of the roots. In conclusion, the systematic position of the genus and its relation to other Pteridophyta, recent and fossil, will be briefly discussed.

With reference to method, it need only be said that we have relied for the most part, though not exclusively, on serial microtome-sections, prepared by the usual paraffin process, and double-stained with safranin and haematoxylin. The small size of the plant and the softness of most of its tissues render it peculiarly amenable to microtome-treatment, and the study of complete series of sections, extending

through the whole length or thickness of the stem has proved of great advantage<sup>1</sup>.

The Guernsey specimens of *Isoetes Hystrix* are small, the extreme length of the leaves not exceeding two inches, while the full diameter of the bulbous stem reaches about half an inch. The upper part of the short stem is covered by the swollen, sporangiferous bases of the living leaves, while the lower portion is enclosed in an armour of the persistent, spinose bases, almost black in colour, of the leaves of previous years. As is well known, the cortex is not, as a rule, thrown off in the terrestrial species of *Isoetes*, or only very slowly, so that the whole stem, or all except the oldest part at the base, remains protected by the spines.

All the specimens we examined had three furrows, separating three well-marked lobes. This is almost constant in the species, though four-lobed specimens have been described. Admirable illustrations of the habit of *Isoetes Hystrix* will be found in the monograph by Motelay and Vendryés, Plates XV and XVI<sup>2</sup>. The appearance of the three-furrowed stem, as seen from below, after removing the roots and spiny leaf-bases, is shown in our Plate XXIV, Fig. 29. The Guernsey plant appears to belong to the variety *subinermis*, of Durieu, as shown by the shortness of the spines (see Fig. 28), though the stems have not usually undergone the early desquamation, stated by Motelay and Vendryés (p. 490) to characterize this variety.

As the plant grows, in nature, the whole of the bulbous portion, including the bases of all the living leaves, is completely buried in the soil, so that only the tuft of narrow, green leaf-blades is exposed to view. Thus no sign of the presence of fructification is visible externally, and we have the rare case of a plant with subterranean organs of repro-

<sup>1</sup> I desire to place on record that the great majority of the preparations, and the whole of the drawings, were the work of my colleague, Mr. T. G. Hill. On the other hand, I assume all responsibility for the theoretical views expressed at the conclusion of the paper.—D. H. S.

<sup>2</sup> For works cited, see the list of literature at the end of the paper.

duction. The conditions under which fertilization and dissemination take place appear to deserve investigation. Possibly earthworms may play some part in the matter.

#### THE STEM.

The general structure of the stem of *Isoetes* has been known for many years. A very accurate account of its main features, as shown in *I. lacustris*, a two-furrowed species, was given by v. Mohl in 1840, and his bold, diagrammatic figures are admirably adapted to give an idea of its peculiarities. From that time onwards our present knowledge of the stem-structure has been gradually built up by the successive labours of Hofmeister, Alexander Braun, Russow, Hegelmaier, Bruchmann, Farmer, and others. Our knowledge is still imperfect as regards the mode of growth at the apex, the differentiation of the tissues, and the nature of the secondary growth. On these points we hope that some new light is thrown by our observations, to which we will now pass on, dealing with the statements in the literature as we proceed.

The short, three-lobed stem of *Isoetes Hystrix* has a deep depression at the apex, at the bottom of which the growing-point is situated. The sloping sides of the funnel-shaped depression are covered by the leaves, becoming successively younger as they approach the growing-point at the bottom of the funnel. In these respects there is no essential difference from the stem of other species, and various figures in the literature will serve to illustrate the general arrangement<sup>1</sup>. The growth of the stem in length is extremely slow, while the cortex, owing to the extension of the primary tissues, and to cambial activity, has a vigorous circumferential increase. It is the extraordinarily stunted form of the *Isoetes*-stem which gives rise to most of its peculiarities.

The stem is traversed longitudinally by a single stele; in its upper part the stele is cylindrical, while lower down the

<sup>1</sup> See, for example, v. Mohl, 1845, Pl. V, Figs. 9 and 10; Hofmeister, 1852, Pl. X, Fig. 1, Pl. XII, Fig. 1, &c.; Wilson Smith, 1900, Pl. XIII, Figs. 3 and 4.



stem it becomes very markedly triangular, as seen in transverse section (cf. Plate XXIII, Figs. 13 and 14), the angles growing more prominent towards the base of the stem. The proportionate length of the cylindrical and triquetrous portions of the stele, though showing individual variations, remains approximately constant in the young and old stems. The transition from the one form to the other is gradual, and the exact limit therefore arbitrary, but roughly speaking, the upper, cylindrical portion occupies from about one-third to two-fifths of the whole length, while the lower, more or less triangular region, extends through the remaining two-thirds or three-fifths. A precisely corresponding change in the form of the stele takes place in the two-furrowed species, only here the basal region is, in transverse section, an elongated ellipse, instead of a triangle (v. Mohl, l. c., Figs. 7 and 8). The projecting arms correspond in position to the furrows, and are due to the abutment of successive root-bases. As new roots arise in acropetal succession, the triangular region (in the three-furrowed species) gradually encroaches on the cylindrical, keeping pace with the general growth in length, and thus maintaining an approximately constant relation between the two regions. Thus every part of the stele is cylindrical in its young condition, and only assumes the triquetrous form with advancing age, as it becomes the seat of root-formation.

The stele gives off numerous leaf-traces, which start almost horizontally, and then, bending upwards, pass out one into each leaf. The whole stele bears leaf-traces, but they are usually only to be recognized on the upper, cylindrical portion; for lower down, the old traces have become more or less completely obliterated, and their continuity has been interrupted by the excessive growth of the secondary tissues through which they pass (cf. Hofmeister, 1852, p. 149).

Before going on to consider the differentiation of the tissues, it is desirable to say something as to the apical growth of the stem as a whole, a point on which there has been much difference of opinion, and which is not yet cleared up. Hofmeister, as is well known, attributed the apical growth to

a single apical cell, and even described its mode of division in considerable detail. At that time, however, the single apical cell was the only type of growing-point which had been investigated, and it was natural that the great morphologist should have endeavoured to refer the stem, at least of any Vascular Cryptogam, to that scheme. Later investigators have generally rejected his explanation. Hegelmaier (1874, p. 497) came to the conclusion that the meristem of the growing-point in *I. velata* and *Duriaei*, at which he worked, had the significance of an apical cell-surface: he appears to regard this initial layer as extending over the whole area of the apex. Bruchmann (1874, p. 570) found in the case of *I. lacustris*, a definite initial group, occupying the middle of the apex. The apex of the stem, however, as shown in his figures (Plate XXIV, Figs. 29–31), bears a suspicious resemblance to a young leaf. Farmer, on the other hand (1890, Plate V, Figs. 13 and 14), working with serial sections, no doubt observed the true apex of the stem, which is comparatively flat. He finds that 'the entire apex of the stem is covered by a columnar layer of cells, which divide chiefly anticlinally, periclinal divisions only occurring at long intervals' (p. 39). He thus practically returns to Hegelmaier's conclusion, and makes no attempt to identify a definite apical group. Van Tieghem (1891, p. 1429) states that the stem grows 'like that of the Lycopods, by a single small mother-cell.' We are not aware on what grounds this statement is made. Our own observations, while by no means decisive as to the mode of growth, show that Hofmeister's opinion is at least defensible. In good transverse sections through the actual apex, we several times detected a large cell, or two large cells, in a central position. Fig. 1 is from a section showing the surface of the growing-point. A pair of large cells, with larger nuclei than their neighbours, is conspicuous at the centre of the apical region. The wall between them is delicate, which favours the idea that the larger of the two may be the actual apical cell, while its slightly smaller companion may be a segment just cut off from it. This was from one of the larger stems.

Fig. 2, from another, younger specimen, shows a transverse section passing immediately below the actual surface of the apex<sup>1</sup>. The large, somewhat triangular cell, with a large nucleus, is strongly suggestive of an apical cell, from which segments have been cut off.

A third transverse series showed a similar predominant cell at the centre of the apex, towards which the surrounding meristem appeared to converge in radial series.

Other specimens, cut transversely, gave no decisive results.

Longitudinal series require the greatest care, in order to determine which is the median section. The apex is so flat that its form gives little or no guidance, and we were only able to satisfy ourselves as to the median plane, by counting the serial sections, and choosing the middle one, i. e. that which is equidistant from the youngest leaves on either side. We much doubt whether, in this species, the point could be determined without the aid of serial sections.

Fig. 3 represents a longitudinal section through the centre of the growing-point. It shows a central cell (*a*) deeper than the rest, and with a large nucleus. On the left a segment appears to have been cut off, and then subdivided by a transverse wall. Fig. 4, from one of the adjacent sections, misses the large cell, but shows at *s* what appears to be a subdivided segment.

It is no doubt possible that the various appearances described, pointing to the presence of a single apical cell, may be accidental, the large cell having no special relation to the general growth. With such a slow-growing apex, it is difficult, or perhaps even impossible, to arrive at certainty. It is, however, a somewhat unexpected fact, that whenever any definite arrangement could be detected, it appeared to favour the hypothesis of an apical cell. Our observations may at least serve to explain, if they cannot establish, the opinion of Hofmeister.

The central cylinder of the stem is derived from a plerome-

<sup>1</sup> These are thin microtome-sections, about  $6\mu$  in thickness, so that the section drawn shows the same cells as at the surface.



like column of tissue, the cell-rows of which converge upwards, towards the central part of the apex. As we follow the cylinder downwards from the apex, in successive transverse sections, we find that it very rapidly assumes mature structure. The leaf-traces, where they abut on the stele, are somewhat more differentiated than the stele itself, and have their xylem lignified at a level where the central tissue is still in a meristematic condition. Immediately below this point, however, and at a distance not exceeding .15 mm. from the apical surface, the differentiation of the stelar wood suddenly begins. The lignification at first extends irregularly across the stele, connecting the leaf-traces together, but in the next section of the series it may be already complete (Fig. 5). Where the differentiation is so nearly simultaneous it is perhaps futile to seek to determine its direction. In some cases, however, a centripetal development can be traced, as shown in Fig. 6, where the outer stelar tracheides are fully formed, while the central tissue is still undifferentiated. At a slightly lower level, the central tracheides are sometimes found partially lignified, while those towards the periphery are completely formed.

The primary tracheides are extremely short, corresponding to the stunted character of the stem, and their chief diameter is usually horizontal. The thickenings on their walls are mostly spiral, but annular and reticulate markings also occur here and there.

Among the tracheides a considerable amount of thin-walled parenchyma is interspersed, the cells of which retain their nuclei, and remain living when the lignified elements between them are already disorganized.

Fig. 5, from the youngest stem at our disposal, which was little more than 2 mm. in diameter, gives as far as possible a representation of the primary structure of the stele, as seen in transverse section. It is however impossible to find the structure in a purely primary condition, for the cambial divisions begin before the primary differentiation is completed. In all the stems examined, whether old or young, tangential

divisions around the periphery of the stele were well marked, at a level above the commencement of lignification of the primary tracheides. These divisions take place in cells immediately outside those which differentiate into the wood of the stele. No demarcation between pericycle and cortex could be detected.

The question of the existence of phloem in the stem of *Isoetes* has been left in a very unsatisfactory condition, so much so that the latest writer on the genus, Wilson R. Smith (1900, p. 227), proposes to drop the application of the word phloem altogether 'until its justification shall be established on physiological grounds.' This seems rather a remote contingency in the case of a genus like *Isoetes*, and it is well that the anatomy does not leave us so entirely in the dark. Mr. Wilson Smith treats the whole of the so-called prismatic zone, i. e. the secondary tissue lying on the inner side of the cambium, and thus immediately surrounding the primary cylinder, as one tissue, and argues against its being regarded as phloem, on the ground that some of its elements may become converted into tracheides. As a matter of fact the 'prismatic zone' within the cambium is sharply differentiated into three kinds of tissue—secondary parenchyma, secondary wood, and the true phloem. As was first detected by Hegelmaier (1874, p. 500), this zone consists of alternating, concentric bands of starch-containing tissue, and of cells without obvious contents. The existence of this differentiation can always be detected, though more obvious in the older stems. The starch-containing cells retain their nuclei, while the phloem-cells soon lose theirs; in young phloem-cells, granules which probably represent the disorganizing nuclei are often present. The presence of an enucleate zone is easily recognized, even apart from other characters, in stained sections. It is usually first differentiated immediately within the cambium, and separated from the primary wood by a layer or two of parenchyma (see Fig. 5). The phloem-elements have an extremely characteristic structure of their cell-walls, which comes out conspicuously in sections deeply stained with haematoxylin. Their walls are

much pitted, the thicker bands of membrane, between the pits, forming a lattice-like reticulum (see Figs. 7 and 12). The pits are often subdivided by fine bars, into smaller areas. Little of the nature of formed contents can usually be detected, but sometimes small, deeply staining globules are found adhering to the walls, and apparently localized at the pits (Plate XXIV, Fig. 16). In the older parts of the stem the phloem is to a great extent obliterated, dense masses of callus-like substance appearing on the cell-walls, and almost filling the cavity (Fig. 17). These masses stain like callus with coralline-soda, but the other callus-reactions tried did not give wholly satisfactory results, and, unlike true callus, these masses are apt to shrink away from the cell-walls. In their deep staining with haematoxylin they agree with the true callus in the sieve-tubes of the foliar bundle.

We have not investigated the more minute histology of the phloem, and thus have not demonstrated the perforation of the thin-walled areas. That may be left to other investigators, but in the meantime, we can scarcely doubt that these enucleate elements, with the characteristic areolation of their walls, and their agreement in various reactions with the sieve-tubes of the leaf, with which, as we shall see, they are continuous, are best to be regarded as themselves representing the sieve-tubes of the stem. In any case we must apply the name phloem to them exclusively, in describing the stem-anatomy.

Russow detected these elements as long ago as 1872 (p. 139) and described them quite clearly. He says:—‘The tabular or shortly prismatic cells have clearly thickened and finely pitted walls, and in transverse section make quite the impression of sieve-tubes or latticed cells in Coniferae: in their function they certainly agree with the bast-elements mentioned: their difference in form from sieve-tubes is explicable by the conditions of growth of the organs in which they occur.’ Considering that Russow worked with herbarium-material, the accuracy of his description is wonderful, and it compares extremely favourably with later statements. He does not,



however, distinguish between the latticed cells and the ordinary intracambial parenchyma. This distinction, as already mentioned, was first drawn by Hegelmaier, but he failed to make out the latticed structure of the 'empty' cells, owing no doubt, as he himself suspected, to defective optical appliances (l.c., p. 503). Farmer (1890, p. 42) confirms Hegelmaier's account of the alternating zones.

Russow regarded the secondary intracambial tissue as phloem, which is no doubt correct, but with the limitation that only the latticed elements, forming part of that zone, are the true phloem. The parenchyma alternating with the phloem-bands is best regarded as secondary ground-tissue, not as phloem-parenchyma, for there is no reason to associate it with the phloem any more than with the secondary xylem, which forms part of the same region.

Speaking generally, the whole of the phloem in the stem of *Isoetes* must be regarded as secondary, for it belongs to the tissues cut off on the inner side of the cambium. It is not usually possible to identify any *primary* phloem with certainty. In fact the tangential divisions begin so early that it would scarcely be practicable to distinguish between primary and secondary structures, at the periphery of the stele. In certain cases, however, the cambium at its first origin is a normal one, the phloem-elements lying on its outer side, and in these instances the extra-cambial phloem may be reasonably regarded as primary. (See Fig. 8.) It may here be pointed out that the tissue in immediate contact with the primary wood, which Hegelmaier inclined to regard as phloem (1874, p. 502), is certainly not of that nature, as it is always parenchymatous, with none of the characters of the phloem.

Not infrequently the cambium, at its first origin, cuts off a few secondary xylem-elements in contact with the primary wood (Fig. 7,  $x^2$ ). In one stem the only secondary wood found was in this position. These indications of a normal cambial development are of some interest, as suggesting the possibility that the anomalous secondary growth of *Isoetes* may have been derived from a more regular mode of thickening,

such as obtained in *Sigillaria* and most *Lepidodendreae* of which the structure is known. On the whole, however, the process in *Isoetes* more nearly resembles that occurring in *Lepidophloios fuliginosus*, the most irregular of the *Lepidodendreae* in its secondary growth.

The cambium, in *I. Hystrix*, arising in the tissue just outside the xylem-cylinder, continues its activity indefinitely, producing parenchyma and phloem, and a variable amount of secondary xylem, on its inner side, and secondary cortical parenchyma only, towards the exterior. Where the cambium is at first a normal one, with phloem on its outer side, its activity is of short duration, and it is immediately replaced by a new generative layer, arising further to the exterior. Usually the cambium, from its first origin onwards, is anomalous, in so far as it produces phloem towards the interior exclusively. There is no regular formation of successive cambial layers: the same layer may apparently continue active throughout the secondary growth. In two cases, however, a new cambium, internal to the first, was observed, arising by the division of secondary parenchymatous cells just outside the primary wood. As this internal cambium did not exist nearer the apex, the presumption is that it was really of later origin than the more external zone. The activity of the two cambial layers had produced some crushing of the elements between them. The inner cambium, like the outer, may produce all three tissues—phloem, wood, and parenchyma—on its inner face.

The active cambial layer can be readily identified by its narrow, tabular cells, containing dense protoplasm, and each with a large nucleus. In this species, at any rate, the cambial cells do not contain starch, as Wilson Smith maintains to be the case in the species investigated by him (p. 227).

The alternating, though not very regular zones, of parenchyma and phloem, are a constant feature of the intracambial tissue. In an old stem we counted from eight to ten layers of each.

On the other hand the development of the secondary wood

is extremely variable, though it is always present to a greater or less extent. In one stem of fair size we found, as already mentioned, no other secondary tracheides than the few occurring immediately outside the primary wood. In other stems, one or more irregular bands of secondary wood are differentiated here and there between the phloem-zones (Fig. 10). Sometimes the secondary tracheides are isolated (e.g. Fig. 9); in other cases they form considerable aggregations, as shown in Fig. 11, from a specimen in which there was an almost continuous band of secondary wood round the stem. There is no doubt that the elements of the secondary, like that of the primary wood, are true tracheides, which not only acquire lignified thickenings on their cell-walls, but lose their living contents. The statement made in the case of *I. lacustris*, that these elements contain protoplasm and starch, certainly does not hold good for *I. Hystrix* (Farmer, 1890, p. 42; Campbell, 1895, p. 291). The secondary tracheides, where they occur, are perfectly sharply differentiated, and agree in all important respects with those of the primary wood.

The extracambial secondary cortex does not attain such a great development in *I. Hystrix*, where the primary cortex is not exfoliated, as appears to be the case in some other species. The thickness may even be less than that of the intracambial secondary tissue, especially low down the stem. There are some indications that the secondary cortical cells may undergo further divisions after they are cut off by the cambium.

Thus the secondary growth of the *Isoetes* stem is on the whole anomalous, if we take the typical Gymnospermous or Dicotyledonous stem, or that of most of the Lepidodendreae, as the standard of comparison. Its peculiarity consists in the fact that as a rule, though not invariably, the true phloem, as well as the secondary xylem, is formed on the inner side of the cambium, accompanied by secondary ground-tissue, while on the outer side, ground-tissue alone is produced. The mode of growth is thus roughly comparable to that of the Monocotyledons, such as *Dracaena*, *Tamus*, or *Aristea*, with secondary tissue-



formation. The Monocotyledonous analogy was first clearly pointed out by Russow (1872, p. 158), though it had already been suggested, on less definite grounds, by v. Mohl (1845, p. 125) and Hofmeister (1852, p. 159). Later authors have usually referred to this analogy, which is of course of no value as an indication of affinity.

Most investigators have regarded the stele of *Isoetes* as built up of leaf-traces, and as having no cauline portion. This is the view of Hofmeister, Russow, Sachs, De Bary, and among recent writers, of Farmer and Campbell. Farmer, however (1890, p. 40), allows that the distinction lies 'rather in the mind of the investigator than in the actual object before him.' It seems to us to be somewhat arbitrary to speak of a vascular cylinder as built up of leaf-traces when it is manifestly impossible to refer its constituent elements to the particular leaf-trace to which they belong. *Isoetes* appears to have a cauline stele just as any monostelic Lycopod has, only in the former the stele is much shorter than in most Lycopods, and the leaf-traces joining it more crowded. The stelar wood serves to join up the xylem of the leaf-traces, but does not belong to one trace more than to another, and in structure it differs obviously from that of the leaf-traces. We therefore prefer to adopt the view of Hegelmaier and Bruchmann<sup>1</sup> that the xylem-cylinder is cauline. The phloem is certainly cauline but hardly comes into the question, as the primary phloem can so rarely be identified. The view of the central cylinder as cauline of course applies to the adult stem only; in embryonic stages, according to all investigators, the construction of the vascular system from the union of definite leaf-traces is indisputable. Similar differences between the embryonic and adult stem obtain in certain species of *Lycopodium*.

Russow maintained that his 'phloem,' i. e. the intracambial zone generally, was continuous with the phloem of the leaf-traces. Some other authors have disputed this, e.g. Mr. Wilson Smith in his recent paper (1900, p. 227). Our own observa-

<sup>1</sup> Hegelmaier, 1874, p. 505; Bruchmann, 1874, p. 570.

tions completely confirm Russow's statement, but give it greater precision. The phloem of the leaf-traces (as to the nature of which there is no longer any doubt) passes over directly into those latticed elements of the intracambial zone, which alone constitute the true phloem of the stem (Fig. 12). At the point of junction, transitional elements occur, which belong equally to the leaf-trace and to the cauline phloem.

As we follow the stele downwards, we find that its primary wood becomes more and more disorganized, the tracheides breaking down so completely that they must become perfectly functionless. The parenchymatous elements of the wood remain living, and appear even to grow in length, forming a trabecular network, in the meshes of which the remains of the disorganized tracheides are scattered. Possibly a pull may be exerted on the central wood by the leaf-traces, when stretched in consequence of secondary growth (Farmer, 1890, p. 41), but if so it has little effect in enlarging or distorting the cylinder, which is smaller in the lower than in the upper part of the stem, and usually shows little change from its normal cylindrical form.

The change to the triangular section of the basal part of the stele, as above explained, is due to the development of the adventitious roots. In *I. Hystrix*, the roots are developed along three lines, corresponding to the three furrows. The root-bearing furrows start from the centre of the base of the stem, and run outwards and upwards, dying out below the region of the living leaf-bases (see Plate XXIV, Fig. 29). The three prominent arms, seen in transverse sections of the lower part of the stele, correspond in position to the three furrows.

The structure of the basal part of the stele in *Isoetes* depends so completely on the distribution and development of the roots that a few words on this subject are necessary here, though the structure of the roots themselves will be deferred till later.

The order of succession of the roots of *Isoetes* appears to have been first correctly made out by Hofmeister. We worked

out the order for ourselves in *I. Hystrix*, and as the published accounts are not altogether clear, we have thought it worth while to illustrate the subject by the two accompanying diagrams (Figs. 11 and 12 in the text), which were made from camera-lucida drawings, so that the position of the roots is exact. The relative age of the roots could be determined by their state of development, the oldest root-traces having their vascular tissues more or less obliterated, while the youngest were still wholly meristematic, and all intermediate stages

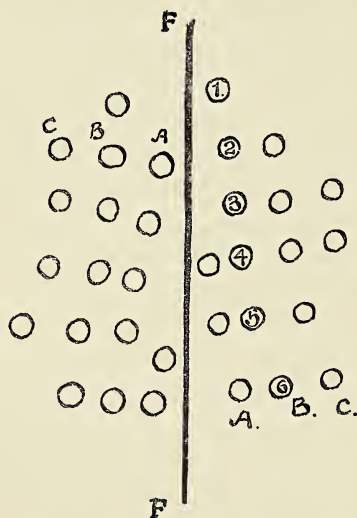


Fig. 11. Diagram of the root-succession in *I. Hystrix*, as shown in a tangential section of the stem, at right angles to a furrow.

were represented. Both sections pass through the region of the root-bases. Fig. 12 is from a transverse section through the base of the stem, while Fig. 11 represents a tangential section, from the side of the stem, cut at right angles to one of the furrows.

The succession of the roots of *Isoetes*, like that of most other adventitious roots, is an acropetal one. Yet new roots are constantly arising on the base of the stem. The apparent contradiction is thus explained: the roots, in our species, are arranged in three sets, corresponding to the three furrows. We will fix

our attention on one set. The roots are arranged in several parallel series, on each side of the median line of the furrow (*F* in the diagrams); the whole set of roots extends from near the centre of the basal surface of the stem outwards, and for a certain distance up the flank. In each series the succession of the roots is acropetal, the oldest being nearest the centre of the base of the stem, and the youngest highest up on the flank. But, at the same time, new series are being



started, each lying nearer the median line of the furrow than its predecessor. Hence we may at any time find new roots arising near the centre of the basal surface; these belong to new rows which have only just started. With reference to the whole stem, the succession of the roots in each series is thus acropetal, while the succession of the series themselves is centripetal, with reference to the centre-line of the furrow to which they belong. In Figs. 11 and 12 the succession

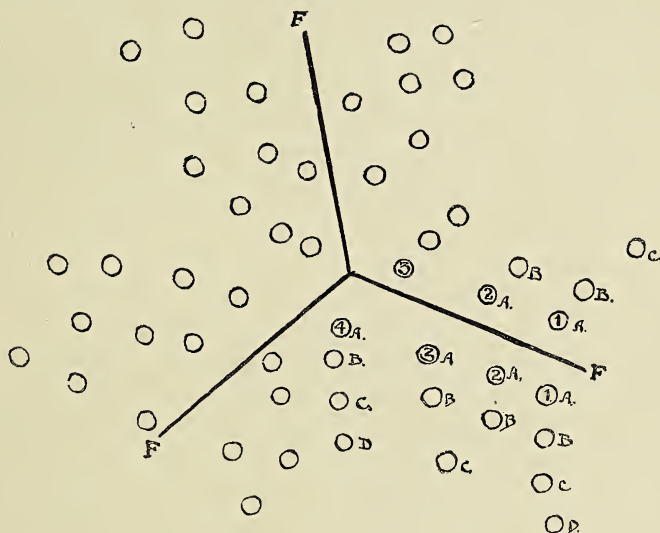


Fig. 12. Diagram of the root-succession, as shown in a transverse section from the base of the stem. For description see text.

within each series is represented by numbers, No. 1 being the *youngest* root shown in its series; the succession of the series themselves is represented by letters, *A* being the *youngest* series of its set. There will usually be two, about equally young series, one on each side of the median line of the furrow. It would rarely happen that the same series could be traced through its whole course. The older series would usually be obliterated near the base of the stem, while the younger series would be incomplete towards the apex. It

will be noticed that in Fig. 11 (which being from the side of the stem, shows only the younger roots of those series which reach so far) an intermediate row on each side is the longest. This is so, because the younger series, towards the middle, are not yet carried so far up, while the older series, towards the outside, were completed while the whole root-bearing region was shorter.

The older and outer series of each set get pushed further and further away from the centre-line of the furrow by the growth of new series of roots, and of the cortical tissue between them.

The three prominent arms of the stele, in its lower portion, are simply due to the abutment on the vascular cylinder of the three sets of roots. Each root, as is well known, arises from cortical tissue<sup>1</sup> just outside the cambium. It is thus separated at its origin, from the wood of the stele, by all the intracambial tissue which has been formed. This tissue becomes partly differentiated into wood, so as to connect up the new root with the conducting tissues of the plant generally (Fig. 15). Each new vertical series of roots necessarily arises a little further out from the stele<sup>2</sup> than its predecessor, and its abutment adds a little to the length of the arm to which it belongs. We thus obtain a kind of sympodium, the wood at each angle being built up of the successive root-bases. The original cylindrical xylem-cylinder remains, more or less unaltered, in the middle of the whole structure. The same thing is going on at the base of the stem, as new series of roots start there. Their bases join on successively to the bottom of the stele.

It has long been known that the base of the stem of *Isoetes* has a slow downward growth, a fact which much exercised the earlier writers. Von Mohl (1845, p. 125) discusses, and leaves undetermined, the question whether *Isoetes*

<sup>1</sup> We use the word *cortical* because the attempt to delimit a pericycle appears quite hopeless in the case of the *Isoetes*-stem.

<sup>2</sup> This will be evident on comparing Text-fig. 11 with Plate XXIII, Figs. 14 and 15.

possesses a 'caudex descendens,' comparable to the tap-root of a Dicotyledon; Hofmeister (1852, p. 159) lays great stress on the Dicotyledonous analogy. As a matter of fact, the downward growth is due to the cambium, which at the base of the stem appears to form secondary parenchyma only; the wood which is added at the bottom of the stele, and which remains functional when the primary wood higher up is disorganized, is entirely built up by the sympodium of root-bases. There is thus no real similarity to the apical growth of a tap-root, though there is a physiological analogy, in so far as the *Isoetes* arrangement allows of the constant development of new roots at the base of the axis. The furrows owe their origin to the cambial growth being checked along the lines where the root-bases are attached, while it attains its maximum between them.

#### THE LEAVES AND LEAF-TRACES.

The structure and development of the leaves of *Isoetes*, and of *I. Hystrix* in particular, have been admirably elucidated by Kruch (1890), whose accurate investigations have been confirmed in almost every point by our own observations<sup>1</sup>. A short statement of our results will therefore suffice.

The mature leaf of *Isoetes Hystrix*, as seen in a transverse section of the lamina, i. e. above the sheathing base, has the structure represented diagrammatically in Plate XXIV, Fig. 18. The sectional form is roughly triangular, with the broad base of the triangle forming the upper or ventral surface. The mesophyll is traversed by four large intercellular spaces, alternating with which are four strands of mechanical fibres (Fig. 23), two at the ventrolateral corners, one at the middle of the ventral surface, and one at the dorsal angle. In this species the intercellular spaces are roofed in by the epidermis only; the stomata are limited to those bands of the epidermis

<sup>1</sup> For a translation of a great part of the Italian text of Dr. Kruch's paper, we are indebted to the kindness of Mr. L. A. Boodle, F.L.S.



which cover in the cavities. Each of these bands bears three or four alternating longitudinal rows of stomata.

Longitudinal sections show that the intercellular cavities are partitioned at intervals by transverse diaphragms, one cell thick in the middle, but thicker where they abut on the surrounding mesophyll, which constitutes the assimilatory tissue. The middle of the leaf is traversed by the vascular bundle, surrounded by a small-celled parenchyma. The structure of the vascular bundle is obviously collateral (Fig. 19).

About the middle of the bundle is a well-marked, circular, intercellular space (Fig. 19, *px*), and on either side of this there is often a smaller space of similar form. On the inner, ventral side of the intercellular spaces are the tracheides, not more than about eight or nine in number, forming an irregular transverse band, and usually separated from one another by xylem-parenchyma. The thickenings of these tracheides are, as a rule, annular (Fig. 22). On the outer side is the phloem, somewhat crescentic in sectional form (Fig. 19, *ph*), with the horns of the crescent occupied by the smaller elements. In the mature bundle it appears to be the case, as stated by Kruch, that the sieve-tubes are limited to the lateral horns of the crescent, for none were found, at maturity, in the median position. The elements of the phloem, where fully developed, have thick, cellulose walls, so as to have the appearance of mechanical elements, though only sieve-tubes and phloem-parenchyma are represented.

Both the central and lateral cavities of the bundle are surrounded by an endodermis, with suberized radial walls, showing the characteristic dark dots. This curious fact was first discovered by Russow (1872, p. 140), and is well illustrated by Janczewski (1882, Pl. IV, Fig. 8). Kruch (1890, p. 61) did not find the endodermal structure in his specimens; it is most distinctly present in ours, and the radial walls resist the action of sulphuric acid. Strasburger has already pointed out (1891, p. 464) that the endodermal differentiation varies with the conditions. Our observations show that it may be characteristically present in a purely terrestrial

species<sup>1</sup>. As Kruch pointed out, the wall lining the cavity is distinctly suberized. As Janczewski first showed (1882) and Kruch demonstrated in detail (1890, pp. 61, 70), the canals in the foliar bundle of *Isoetes* represent disorganized xylem-elements; the large central canal takes the place of the actual protoxylem, the first-formed row of tracheides (Fig. 20, *px*). These are of rather large size, with transverse walls, which persist for a long time, and are pulled widely apart by the growth in length of the leaf, so that in a microtome-series from a partly-developed leaf, one wall was seen about every hundred sections. These transverse walls or diaphragms are lignified and reticulated, and are perforated, at least when old (Fig. 21; cf. Fig. 22, *px*). Strasburger has shown that these canals in the bundle of *Isoetes* contain water, so it appears that they continue to perform tracheal functions. The tracheides corresponding to the lateral canals develop rather later, and so become less disorganized. In the upper part of the leaf they usually persist as tracheides, so that here there is only the one median canal, and it is then only around the latter that the endodermis is differentiated. The first-formed tracheide, as we have seen, is the outermost, so that here the wood is developed centripetally (with reference to the centre of the stem), as Russow states.

The protophloem, which is differentiated earlier than the protoxylem, forms a short band of apparently empty elements, with deeply-staining cellulose walls (sieve-tubes according to Kruch), lying immediately opposite the first tracheide (Fig. 20, *pph*). This median phloem becomes obliterated, and it is only the later-developed sieve-tubes of the lateral phloem-bands which remain functional. Kruch (1890, p. 70) and Strasburger (1891, p. 463) state that new sieve-tubes are added to the lateral bands, towards the dorsal (outer) side, and this appears to be the case.

The sieve-tubes, though small and difficult to investigate,

<sup>1</sup> The endodermal markings are not shown in our figures, as the microtome preparations were not favourable for demonstrating them. They were quite clear in sections mounted in glycerine.

appear to have the typical structure. Fig. 24 shows, in vertical section, a transverse sieve-plate, which is thickened by a callus-deposit. Sieve-areas also occur on the lateral walls, and on these also, swollen masses, giving the reactions of callus, are formed (Fig. 25).

Towards the sheathing base of the leaf the structure changes considerably. The mechanical strands die out, the stomata diminish in number and ultimately cease, and the mesophyll here contains little or no chlorophyll. At the level of the glossopodium the four cavities of the mesophyll come to an end. Lower down, two new cavities appear, one on each side, and at the level of the sporangium these cavities take up a position in the wings of the leaf, on either side of the velum. As regards the vascular bundle, the number of xylem-elements increases in the centripetal direction; the three intercellular spaces of the xylem are extremely conspicuous in the region immediately above the base of the ligule. Still lower down, opposite the sporangium, the structure of the bundle undergoes a more important change. No intercellular spaces are formed in the xylem, which here consists of a considerable number of small tracheides, intermixed with parenchyma. Kruch (1890, p. 76) states that the xylem in this region, and in the leaf-traces, is not always entirely centripetal, but that some elements are added towards the phloem. This statement is confirmed by our own observations, which in some cases showed developing tracheides between the first-formed xylem-elements and the phloem. Thus the foliar bundle towards its base tends to pass from exarch to mesarch structure.

The median protophloem is persistent in this part of the leaf, so that the layer of sieve-tubes forms a continuous arc when mature, and is not divided into two lateral groups as in the lamina, higher up.

The base of the deep-seated ligule is surrounded by a very conspicuous sheath of tracheides, as described by many previous writers (see Fig. 27). These tracheides, which are developed early, are especially abundant on the ventral side



of the ligule, at the base of the labium. When the leaf is mature, this sub-ligular xylem, which is not so strongly lignified as that of the foliar bundle, may nearly reach the latter, but we have never observed actual continuity between the two tissues.

The leaf-traces, where they pass through the cortex of the stem, are collateral in structure. Near the stele, the xylem, which is well developed, is made up of elements in close contact with one another, while in the outer cortex the tracheides are separated by parenchymatous cells. The phloem is well marked; the elements composing it, which are separated from the xylem by parenchyma, present all the characteristics of the protophloem of the leaf, in shape, size, thickness of walls, and the decided blue colour which they take up when stained with haematoxylin.

The leaf-traces, where they traverse the outer cortex of the stem, are practically identical in structure with the bundle of the leaf in the region of the sporangium.

Immediately before joining the central wood, the leaf-trace of course shows no phloem; here it is surrounded by a well-defined zone of parenchyma, the outer boundary of which marks the limit between the leaf-trace and the surrounding, growing tissue of the intracambial zone.

The development of the leaf is well known, and may be briefly dealt with. It arises as a dome-shaped mass of tissue, from the superficial layers of the apex, and grows at first towards the centre of the apical depression. The ligule is the first part of the leaf to be differentiated. It arises from a single cell, which grows enormously in size, while the leaf is still quite small. The first divisions in this cell are transverse. Longitudinal growth takes place very rapidly, so that the ligule becomes bent over the apex of the young leaf<sup>1</sup>. The transverse growth of the ligule is also considerable, so that it exceeds the young leaf in width.

The *velum* arises from a band of tissue, the *sella* (saddle)

<sup>1</sup> The development of the ligule and its mature structure are fully described in the papers of Farmer, Campbell, and Wilson Smith.

of A. Braun (1863, p. 569), lying between the young sporangium and the ligule. It is certainly not the case in *I. Hystrix* that it arises as 'a sterilized portion of the sporangium,' as Wilson Smith maintains (1900, p. 242). The first growth from the saddle is in the upward direction; to form the *labium* of A. Braun (Fig. 26). The upward growth soon ceases, and the velum develops in the downward direction, keeping pace with the growth of the sporangium, around which it forms a complete pocket, open only by a narrow crevice at the lower end (Fig. 26). This opening was called the micropyle by Braun. The complete velum is characteristic of the terrestrial species of *Isoetes*. As the velum is perfectly continuous with the leaf at the sides, as well as at the upper end, its development must take place by intercalary growth, proceeding *pari passu* with that of the leaf-base and sporangium.

Practically the whole growth of the leaf is intercalary, the growing region being at first localized at the level of the sporangium, and subsequently extending higher up, above the insertion of the ligule. It is only at the first origin of the leaf that there is any apical growth.

The development of the persistent, spinose leaf-bases of *Isoetes Hystrix* was fully worked out by Alex. Braun (1863, p. 578), whose account we have simply to confirm. The facts are briefly these. A transverse ring of tissue at the base of the leaf, extending up to the lower edge of the sporangium, greatly thickens the walls of its cells, so as to form a continuous armoured belt. The sclerotic belt reaches the dorsal and ventral surfaces, and may include the lower part of the velum, but it does not extend out into the wings, the thin part of which withers away. At certain points the sclerosis extends further upwards, forming the spines. These may be four in number: there are two lateral horns, formed from tissue at the insertion of the wings, a ventral tooth formed partly from the velum and partly from tissue on the opposite side of the sporangium, the remains of which are thus partially enclosed by the sclerotic region, and lastly there may be a dorsal tooth, which

is scarcely developed in our short-spined form. All these spinous structures are firmly seated on the sclerotic belt below. The inner tissue of the leaf-base remains living, and, when the lamina is thrown off, is covered in by a transverse plate of hard black tissue.

The whole structure is called by Braun the phyllopodium, a term which has since been used by Bower in a much more extended sense. In the reduced sterile leaves almost the whole organ is used up to form the persistent scale. The appearance of the ordinary spinose leaf-base is shown in Fig. 28.

#### THE ROOTS.

As regards the apex of the root we have nothing to add to the results of previous observers. In this case there can be no question that the earlier writers (Hofmeister, 1852, p. 136; Nägeli and Leitgeb, 1868, p. 135) were mistaken in referring the growth to a single apical cell, while Kienitz Gerloff (1881, p. 793) was equally in error in regarding the tissue of the apex as 'a completely indifferent meristem.' Bruchmann, Farmer, and Campbell have proved conclusively that the root-apex possesses distinct histogenetic layers. The plerome is perfectly well defined. Bruchmann (1874, p. 556) and Farmer (1890, p. 52) find that it grows by means of a single initial cell<sup>1</sup>. Some of our sections appear to confirm this result in the case of *I. Hystrix*, but we doubt whether it holds good constantly in our species. Beyond the plerome the most distinct demarcation is between inner and outer cortex (Farmer, 1890, p. 51).

It appears that the later growth of the root is intercalary, so that the apex can only be studied with advantage in quite young roots.

Dichotomy of the roots takes place very early, while the apex is still buried in the tissues of the stem.

The anatomical structure of the mature root of *Isoetes* is

<sup>1</sup> Compare also Nägeli and Leitgeb, 1868, p. 134, 'Der Cambiumcylinder ist anfangs immer einzellig.'



well known, and is illustrated, somewhat diagrammatically, in Fig. 30.

The position of the stele is from the first somewhat eccentric; a large intercellular space arises in the cortex, by breaking down of its internal layers, on one side of the stele, while, on the opposite side, continuity between central cylinder and cortex is maintained. As regards the external tissues, the chief point of interest is the presence of short cells—the ‘trichome-initials’ of Bruchmann (1874, p. 566)—in the superficial layer, a point in which the root of *Isoetes* agrees with that of *Lycopodium*. It is from these cells only that the root-hairs, which are very abundant in *I. Hystrix*, take their rise.

The structure of the stele is in all cases monarch. There is a single group of xylem, with a single band of phloem, forming a horseshoe on one side of the wood (Figs. 30, 31). The orientation of the tissues is such that the xylem of all the roots, where they spring from the stem, is turned towards the centre-line of the furrow; the phloem, as well as the intercellular cavity, which always lies on the phloem-side, are directed towards the margins of the furrow.

The stele is surrounded by an endodermis of the usual structure, which is clearly of common origin with the inner cortex. The pericycle is incomplete on the side opposite the middle of the phloem.

The wood consists for the most part of densely spiral or annular tracheides, somewhat reticulated in places. The first tracheide to be differentiated lies on the side of the xylem remote from the phloem, as shown in Fig. 32. From this point the development advances outwards, though not in perfectly regular succession, until the xylem is complete. The protoxylem in the roots of the adult plant is, as a rule, separated by the pericycle from the endodermis; in the first root it appears, according to Bruchmann (1874, p. 565) and Farmer (1890, Pl. V, Fig. 4) to be in contact with the latter layer.

The protophloem is recognizable before the differentiation of the xylem begins, and forms an arc, in immediate contact

with the endodermis, and at the opposite side from the protoxylem (Fig. 32). There is thus no distinction between the monarch strand of the *Isoetes* root, and an ordinary collateral bundle of the endarch type.

Where dichotomy of the root takes place, the plane of division passes through the protoxylem and protophloem. A stele at the base of a dichotomy is shown, in transverse section, in Fig. 34. As the two strands separate from each other they turn through an angle of  $90^\circ$ , so as to direct their protoxylem-groups towards each other. The process is precisely the same as in the roots of a *Selaginella* (Van Tieghem, 1871, p. 99, Pl. 5, Figs. 25 and 26), or in the rootlets of a *Stigmarmaria*. We have not examined the minute structure of the phloem of the root, which appears to resemble that of the leaf.

There are certain statements in the literature as to the roots of *Isoetes* which are not confirmed by our observations. Prof. Van Tieghem (1891, p. 1429) states that 'while the root is descending in the cortex, the structure of its central cylinder is binary, and at first normal; the diametral band of xylem is parallel to the central cylinder of the stem. But gradually one sees the phloem-bundle situated on the side towards the axis of the stem, become more and more reduced, and finally disappear, while the diametral band becomes applied to, and concentrated towards the pericycle<sup>1</sup>.' We can find nothing answering to this in *Isoetes Hystrix*.

However near the stele of the stem the root-trace is examined, its phloem is always limited to the side remote from the wood of the stem. Further, in this region, just as in the more distal parts of the root, the development of the xylem proves that its structure is monarch throughout. Fig. 33 shows a young root-bundle cut close to its junction with the stelar wood of the stem. There is manifestly one protoxylem-group only, and the phloem, though as yet but little differentiated, is clearly limited to the side remote from the proto-

<sup>1</sup> This account differs from that on p. 690 of the same work, where the normal structure in *Isoetes*, like that in *Selaginella*, is explained by abortion of one xylem-bundle, and fusion of the two groups of phloem.

xylem. At this point the primitive tracheides of the root-bundle are extremely short, showing the characters of those of the stele.

Prof. Bertrand (1881, p. 51) figures a root of *Isoetes Hystrix* in transverse section, and describes the roots of *Isoetes* as having a bicentric bundle, 'curved so strongly that some authors have considered these bundles as having only a single centre of development.' His figure clearly shows two protoxylem-groups side by side. We have seen no such structure in the roots of *Isoetes*, except at the point of bifurcation; in every case the protoxylem is first differentiated as a single tracheide. At later stages, where several tracheides have become lignified, it might be possible to mistake some of the later-formed elements for a second group of protoxylem. Probably, however, Prof. Bertrand's interpretation is to be explained in a different way. His figure exactly represents the structure of an *Isoetes* root, at the point of bifurcation, the two protoxylem-groups belonging to the two forks of the dichotomizing bundle. Our Fig. 34 may be compared with Prof. Bertrand's, but ours was drawn from a section where the dichotomy was not quite as far advanced as it appears in his.

The whole of the evidence, so far at least as *I. Hystrix* is concerned, appears to us to leave no doubt that the structure of the root-stele is in all parts a strictly monarch one, and that there is at present no indication that this monarch organization has arisen by reduction from any more complex structure.

#### SUMMARY.

##### I. The Stem.

1. There is some evidence that the apex may grow by means of a single apical cell, as Hofmeister supposed.
2. The stele is not composed of the united leaf-traces, but is best interpreted as a cauline structure, comparable to that of the simpler Lycopods.
3. The differentiation of the primary wood is nearly simul-



taneous over its whole area, but in some cases a centripetal development can be traced.

4. The cell-division of the primary meristem passes over without any interruption into that of the cambium.

5. In some cases the cambium is at first normal, lying on the inner side of the first-formed elements of the phloem, and producing secondary tracheides in contact with the primary wood. In this case a second cambium soon arises further to the exterior. In other specimens the more internal cambium arises after the other.

6. As a rule, the same cambium is active throughout, producing secondary ground-tissue, wood, and phloem, on its inner side, and cortical parenchyma only, towards the exterior.

7. Secondary wood is always formed, though very variable in amount. Its elements are typical tracheides, without cell-contents.

8. Well-differentiated phloem, consisting of enucleate elements, with clathrate pitting on their walls, is always present in the intracambial zone, forming definite, concentric bands, alternating with the secondary parenchyma. The phloem of the stem is continuous with that of the leaf-traces.

9. The stele, cylindrical above, becomes triquetrous below, owing to the abutment upon it of the root-bases, successively developed along lines corresponding to the furrows.

10. The development of the roots in each series is acropetal, while that of the several series belonging to each furrow is centripetal, with reference to the centre-line of the furrow.

11. The downward growth of the base of the stem is entirely due to the activity of the cambium, and to the addition of new root-bases.

## II. The Leaf.

The general structure of the leaf is too well known to need recapitulation.

1. The vascular bundle, which is collateral throughout, has exarch structure in the lamina, the protoxylem lying next the

phloem. The central canal of the bundle, which, with the lateral canals, when present, is surrounded by a true endodermis, represents the primitive row of tracheides. Their transverse walls are persistent, forming perforated diaphragms across the canal.

2. In the base of the leaf, and in the leaf-trace, there are no xylem-canals, and the structure of the bundles is in some cases mesarch, a few tracheides being formed between the protoxylem and the phloem.

3. The phloem contains true sieve-tubes with transverse sieve-plates, and lateral sieve-areas, on both of which callus is formed.

4. The growth of the leaf, except at its first origin, is intercalary.

5. The ligule, which develops extremely early, secretes mucilage when young. The glossopodium is surrounded by a sheath of tracheides, which are most abundantly developed in the base of the labium.

6. The labium and velum are derived from tissue above the sporangium, and not from sterilized sporogenous tissue. The velum shares in the intercalary growth of the leaf-base, and forms a complete pocket round the sporangium, only open by a narrow crevice at the base.

7. The persistent spinose scales are formed by sclerosis of certain portions of the tissue of the leaf-bases, whether fertile or sterile, as described by Alex. Braun.

### III. The Root.

1. The stele of the root has, in all parts, a monarch structure, the differentiation of the xylem beginning with the development of a single tracheide, which lies directly opposite the protophloem. Neither at the base of the root nor elsewhere is there any indication of diarch structure.

2. The apex of the root shows distinct histogenetic layers, as described by Bruchmann, Farmer, and Campbell. The initial groups give rise to the plerome, and to the inner and outer cortex.

The general structure and dichotomy of the root require no recapitulation.

#### CONCLUSION.

The investigation of *Isoetes Hystrix* shows that this terrestrial species differs less from the aquatic forms than might have been expected. The xylem throughout the plant is somewhat better developed, but the difference is not striking; the secondary wood, though a constant feature, is very variable in amount, and seldom plentiful. The leaves, with their numerous intercellular spaces, have an aquatic character, shown especially by the formation of the canals, with endodermal boundaries, in the xylem. This curious feature strongly suggests an aquatic adaptation, and it is singular to find it in a terrestrial plant. The whole structure indicates clearly that *Isoetes Hystrix* is in no way a primitive form, but rather represents an aquatic or amphibious type, which has become secondarily adapted to life on land.

While we thus find aquatic characters in a terrestrial species, it is interesting to recall the fact that terrestrial characters also appear in some of the aquatic species. Thus, A. Braun (1863, p. 587) points out that a large part of the genus, including several submerged forms, possesses stomata on the leaves.

We are thus naturally led to regard the genus *Isoetes* as a group that has long hovered on the limit of terrestrial and aquatic life, some of the forms becoming wholly submerged, while a few have definitely betaken themselves to dry land, a large proportion leading a more or less amphibious existence<sup>1</sup>.

For this reason, among others, we cannot accept Mr. Wilson Smith's conclusion (1900, p. 323) that the genus *Isoetes* represents 'a more primitive form of sporophyte than any other vascular plant.' The group has clearly undergone

<sup>1</sup> In Baker's Fern-Allies, out of the forty-nine species described, nine are classed as aquatic, nine as subaquatic, twenty-nine as amphibious, and only two as terrestrial.



reduction (in relation to aquatic habit) from some more complex type, and probably from some very highly organized form of Lycopod, as indicated by the secondary growth, the marked heterospory, and the somewhat complex organization of the leaves and of the root-bearing portions of the axis.

Our investigations have convinced us very strongly of the close Lycopodinean affinities of the genus, and of the absence of any special relationship to the Ferns. It will be necessary to consider briefly the points bearing upon this question. The Filicinean affinities of *Isoetes* have been maintained principally by Vines (1888), Farmer (1890), and Campbell (1891 and 1895).

So far as the asexual plant is concerned, there can be no question that the sum of characters points clearly to Lycopodinean affinities, while, with the single, very doubtful exception of the velum, there is no single point in which the Ferns are approached. The habit of the plant, with its crowded, simple and narrow leaves, is more suggestive of Lycopods than of any other group. The fact that the stem is stunted appears to us to have no bearing on the question of affinities. Elongated and shortened forms of stem occur both in Lycopodiales and Filicales, and throughout the Vegetable Kingdom we find the greatest variety in this respect among closely related plants, and often within the limits of a single genus. Neither is the stunted form of stem by any means specially characteristic of the Ferns. The usually unbranched habit (which is not without exception) is no doubt correlated with the shortened form of stem <sup>1</sup>.

The anatomy of the stem, with its solid stele, from which densely crowded, small and simple leaf-traces pass off, is just what we should expect to find in a stunted Lycopod, and bears no real resemblance to anything in Ferns, though a general similarity between the simplest forms of any two groups is easy enough to find. Secondary tissue-formation is a character

<sup>1</sup> It should be remembered, however, that there is some evidence that many of the Palaeozoic Sigillariae branched little or not at all.

of very little taxonomic value, and we know, chiefly through Williamson's researches, that it was of common occurrence in all groups of Vascular Cryptogams during the Palaeozoic period. The anomalous character of the secondary growth in *Isoetes*, however, agrees somewhat more nearly with that in certain fossil Lycopods, than with the process as known in any of the Filicales.

The discovery of Bruchmann that the base of the stem in *Selaginella spinulosa* (Bruchmann, 1897, p. 39) shows an indefinite growth in thickness, is of great interest, as affording a new link between that genus and *Isoetes*. In this species of *Selaginella* the base of the stem is persistent, and is the only seat of root-formation. The development of secondary wood and parenchyma at the base of the stem is closely connected with the formation of successive new roots in the same region. The conditions, in fact, are closely comparable to those which prevail at the base of the stem in *Isoetes*. The minute histology of *Isoetes* is peculiar, for the tissue-elements have been modified in relation to the habit. They show no approach to the structure of the corresponding tissues in Ferns.

In their whole structure, and especially in their monarch stele, the roots of *Isoetes* agree exactly with those of *Selaginella* and with the 'rootlets' of the Palaeozoic *Stigmaria* (subterranean organs of *Lepidodendron* and *Sigillaria*). So far as the dichotomous branching of the roots is concerned, the agreement extends to all known Lycopodiales. The apical structure of the *Isoetes* root agrees most nearly with that in the genus *Lycopodium* (Bruchmann, 1874). Taking the sum of the root-characters, they are throughout so typically Lycopodinean, that even regarded by themselves they would go a long way to establish affinity. Against this we have to set the fact that in a few species of *Ophioglossum* the roots are monarch, probably by reduction, and are said to be dichotomous in their mode of branching. In other species of the genus *Ophioglossum*, and in the majority of the family Ophioglosseae as a whole, the roots are diarch or polyarch in

structure, and their branching monopodial<sup>1</sup>. If the peculiar characters of the root in certain species of *Ophioglossum* have any taxonomic significance (a doubtful point, which we cannot discuss here), they may indicate a certain approach to the Lycopods generally, but are no evidence of any special relation to *Isoetes*. The mode of origin of the roots of *Isoetes* has been compared to that in Marattiaceae, but there is no real agreement, for the *Isoetes* roots do not arise near the apex, or from the apical meristem, but are always limited to the lower part of the stem, and spring from a secondary tissue<sup>2</sup>. On the other hand, as already pointed out, they agree in these respects with the roots of such species of *Selaginella* as *S. spinulosa*.

Passing on to the leaves, and first considering their anatomy, we find that the single bundle, traversing the narrow leaf without branching, is in itself a Lycopodinean character, though a similar simple arrangement no doubt occurs in a few extremely reduced Filicales. The collateral structure of the bundle is unlike that in living Lycopods, so far as at present investigated, but agrees with the structure of the foliar bundles in the fossil Lepidodendreae. The agreement with the latter is far closer than with the large and branched collateral bundles in the leaf of *Ophioglossum*. In other respects the anatomy of the leaf in *Isoetes* affords no taxonomic indications. The development of the leaf almost entirely by intercalary growth, is, as Wilson Smith has pointed out (1900, p. 333), a marked Lycopodinean character, and forms a striking contrast to the apical foliar development of Ferns.

The general morphology of the leaf, i. e. of the sporophyll, is however of greater importance than any other character. The single large sporangium, seated on the upper side of the sporophyll, near its base, in position, development, and structure is typically Lycopodinean, and has no analogy with

<sup>1</sup> See Boodle, 1899, on roots of *Ophioglossum*, and especially on monopodial branching in those of *O. pendulum*.

<sup>2</sup> The adventitious roots which grow down through the cortex in certain species of *Lycopodium* (e. g. *L. squarrosum*) appear to afford a better analogy with those of Marattiaceae.



anything in Ferns. *Isoetes* is in fact, in this respect, the nearest living representative of the Palaeozoic *Lepidodendreae* (Bower, 1894); the form and large size of the sporangium, its attachment along the whole of its base to the median line of the ventral foliar surface, the presence of trabeculae, the heterospory, and the relation to the ligule, are all points in which there is a remarkably close agreement between *Isoetes* and *Lepidostrobus* (Maslen, 1899). This complete correspondence, at once so obvious and so exact, cannot be invalidated by a supposed analogy (remote, at the best) with the ventral spike of *Ophioglossum vulgatum*.

As regards the ligule, its homology with that of *Selaginella* or of the *Lepidodendreae* would seem manifest. Farmer (1890) has endeavoured to dispute this, on the grounds that in *Isoetes* the ligule develops from a single cell, in *Selaginella* from a group of cells, and that it arises earlier, and is more elaborately developed in the former genus than in the latter. Harvey Gibson (1896) has shown that in the two latter points there is no marked difference between the two genera. The distinction as regards the number of initial cells holds good, but obviously affords no argument against homology in view of the close agreement in all other respects.

The velum of *Isoetes* has been compared with the indusium of a Fern, and regarded as forming the envelope of a 'monangic sorus,' as in the case of *Lygodium* or that of the megasporangium of *Azolla*. No Fern, however, is known in which the sporophyll produces a *single* 'monangic sorus,' and that in the ventral position. Until recently the velum of *Isoetes* was in so far a difficulty, that it appeared to be an isolated case among the Lycopodiales. Quite lately, however, one of us has found that in a Palaeozoic Lycopodinean cone, otherwise identical with a *Lepidostrobus*, each sporangium, whether containing microspores or megaspores, became enclosed in an integument, growing out from the sporophyll<sup>1</sup>. The integument in the fossil cone differs

<sup>1</sup> See Scott, *Studies in Fossil Botany*, 1900, p. 180. A short account of this fructification (*Lepidocarpon*) has been communicated to the Royal Society, and a full description is in preparation.

considerably in detail from the velum of *Isoetes*, but is sufficient to prove that the formation of a sporangial envelope is nothing foreign to the Lycopodiales. Mr. Kidston found some indications of a velum, like that of *Isoetes*, in the cone of *Sigillaria*, but the specimens not having their minute structure preserved, the conclusion to be drawn is somewhat uncertain.

One point, respecting the sporophyte, remains. In *Isoetes* most leaves are fertile, and there is no special strobilus. This fact scarcely affects the question of affinity, for we have almost the same conditions in some species of *Lycopodium*, i. e. *L. Selago*, where also no strobilus is differentiated, and there is a gradual transition from sporophylls to vegetative leaves. In the case of *Isoetes*, we much doubt whether this is a primitive character, for the plant altogether suggests, more than any other genus now living, the reduced successor of some of the gigantic Palaeozoic forms of Lycopodiales, all of which, so far as we know, were strobiloid.

The proposed transference of *Isoetes* from Lycopodiales to Filicales thus finds no support from the characters of the mature sporophyte. The prothallus and embryo lie beyond the scope of our present work, but we must shortly refer to their bearing on the main question.

As regards the prothallus, recent investigations have broken down the distinction formerly believed to exist between *Selaginella* and *Isoetes*, for the supposed differentiation of prothallus and endosperm in the former genus has proved to be illusory, and in reality development proceeds in a similar way in both genera.

The archegonia of *Isoetes* have been compared to those of the Eusporangiate Ferns, and no doubt with good reason, but the comparison is of no weight as against Lycopodinean affinities, for in the genus *Lycopodium* itself, as Campbell has well pointed out, 'the sexual organs closely resemble those of the eusporangiate Ferns and *Equisetum*' (Campbell, 1895, p. 466). Neither does there appear to be any definite distinction between the archegonia of *Isoetes* and those of *Selaginella*.

A point on which stress is laid by Campbell is the presence of large, multiciliate spermatozoids in *Isoetes*, differing much from the small, biciliate spermatozoids of *Lycopodium* and *Selaginella*, and resembling those of Ferns and Equisetaceae. This seems to us the one character in which *Isoetes* really agrees with the two latter classes, rather than with the Lycopodiales as at present known. We have no desire to minimize the importance of this fact, but it cannot, as it seems to us, count for much against the aggregate of Lycopodinean characters.

The embryology has also been much relied on by the advocates of Filicinean affinities, but the resemblance of the embryo of *Isoetes* to that of Ferns appears a remote one. In Ferns the cotyledon and stem arise from the epibasal half of the embryo, the root and foot from the hypobasal half, and all the organs are, as a rule, marked out extremely early. In *Isoetes* the root arises, with the cotyledon, from the epibasal half, while the lower portion produces the foot only. The stem is not one of the primary structures of the embryo at all, but appears quite late, between the cotyledon and the root. *Botrychium*<sup>1</sup> appears to resemble *Isoetes* in the origin of the root from the same half of the embryo as the stem; it differs, however, from our genus, in the fact that the stem precedes the cotyledon in its development. It may be doubted whether the *Isoetes*-embryo, apart from the absence of a suspensor, does not agree quite as nearly with that of a *Lycopodium* as with the embryo of a Fern. The suspensor, however, is known to be a very inconstant feature in other groups of plants; in some Leguminosae, for example, it is altogether absent, while in others it constitutes almost the whole of the embryo at an early stage.

Taking the whole of the known characters of *Isoetes*, we see no reason to depart from the view, which for half a century has generally prevailed among botanists, that *Isoetes* is a true Lycopod. The recent ingenious attempts to connect the genus with the Filicales have not rested on any new discoveries, but have probably been unconsciously influenced by

<sup>1</sup> Jeffrey, 1897.



the desire to find a heterosporous representative of the Eusporangiate Ferns.

Mr. Wilson Smith, in his recent paper, has stated with much force the arguments for the Lycopodinean affinities of *Isoetes*. In addition to points which we have mentioned above, he calls attention to the extremely early development of the sporangium in *Isoetes*, to its multicellular archesporium, and its indehiscent character.

As to the position of *Isoetes* within the Class Lycopodiales, the genus appears to have some real affinity with *Selaginella*, though not close enough to render it desirable to keep them in the same Family. The relationship of *Isoetes* to the Lepidodendreae is probably a nearer one<sup>1</sup>. It may possibly turn out that the curious Triassic genus *Pleuromeia*, of which we have recently had a full description from Count Solms-Laubach (1899), may have been in some respects intermediate between the arborescent Palaeozoic Lycopods and the reduced genus *Isoetes*. We have not thought it necessary to discuss certain resemblances which have been suggested between *Isoetes* and the Monocotyledons. Much further evidence will be required before these apparent points of similarity can be accepted as any indication of affinity.

<sup>1</sup> On this point compare Bertrand Cornaille et Hovelacque, Remarques sur la Structure des *Isoetes*. Saint-Étienne, 1897.

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EXPLANATION OF FIGURES IN PLATES  
XXIII AND XXIV.

Illustrating Messrs. Scott and Hill's paper on *Isoetes Hystrix*.

PLATE XXIII.

Fig. 1. Apical meristem of stem, in superficial transverse section. *a*, two large central cells, of which one may be the apical cell and the other its segment. × 576.

Fig. 2. Part of a similar section from another apex. *a*, apparent apical cell. × 610.

Fig. 3. Apical meristem of stem in median longitudinal section. *a*, apparent apical cell. × 550.

Fig. 4. Adjacent section to the last. *s*, segment-cells. × 550.

Fig. 5. Transverse section of the upper part of the stele, from a young stem. *x*, primary wood; *lt*, leaf-trace; *cb*, cambium. The *enucleate* cells between cambium and wood belong largely to the phloem. × 207.

Fig. 6. Transverse section of the wood of the stele, cut near the apex. *x*, xylem-elements; the differentiation has not yet reached the centre. From a larger stem than Fig. 5. × about 200.

Fig. 7. Peripheral tissues of the stele, in transverse section. *x*, part of primary



xylem;  $x^2$ , secondary xylem, in course of differentiation;  $ph$ , phloem, showing clathrate cell-walls;  $cb$ , cambium.  $\times 442$ .

Fig. 8. Similar section, from another stem, at the commencement of secondary growth.  $x$ , primary xylem;  $cb$ , cambium;  $ph$ , phloem, lying *outside* cambium.  $\times 300$ .

Fig. 9. Secondary tracheide,  $x^2$ , in longitudinal section, isolated among cells of the secondary parenchyma. The primary wood is on the side  $X$ .  $\times 360$ .

Fig. 10. Secondary tissues in longitudinal section, from an old part of stem.  $X$ , direction of primary wood;  $ph^2$ , obliterated secondary phloem;  $x^2$ , secondary xylem;  $cb$ , cambium;  $c$ , secondary cortex.  $\times 180$ .

Fig. 11. Transverse section, showing well-developed secondary xylem,  $x^2$ .  $cb$ , cambium;  $X$ , direction of primary wood.  $\times 325$ .

Fig. 12. Secondary xylem ( $x^2$ ) and phloem ( $ph^2$ ) in transverse section. The latticed structure of the phloem cell-walls is well shown.  $L.T$ , phloem of leaf-trace, continuous with that of stem;  $X$ , direction of primary wood.  $\times 340$ .

Fig. 13. Diagrammatic transverse section of the upper, cylindrical part of the stele.  $x$ , primary wood;  $ph^2$ , region in which secondary phloem;  $x^2$ , that in which secondary xylem is developed;  $cb$ , cambium;  $c^2$ , secondary cortex;  $lt$ , leaf-traces.  $\times 68$ .

Fig. 14. Diagrammatic transverse section of the lower, triquetrous part of the stele.  $rt$ , adventitious roots; other letters as before, but no secondary xylem present.  $\times 22$ .

Fig. 15. Transverse section, showing one arm of the triquetrous part of stele.  $x$ , primary xylem, much disorganized;  $cb$ , cambium;  $rt$ , root-traces, of which several are shown;  $ph$ , phloem, and  $x$ , xylem, of a root-trace.  $\times 213$ .

#### PLATE XXIV.

Fig. 16. Phloem ( $ph^2$ ) from stem, showing pitted cell-walls, and beginning of formation of callus (?).  $\times 576$ .

Fig. 17. Obliterated cells of phloem ( $ph^2$ ).  $\times 576$ .

Fig. 18. Diagrammatic transverse section of lamina of leaf, showing intercellular cavities, stomata, fibrous strands, and vascular bundle.  $x$ , xylem;  $ph$ , phloem;  $Gc$ , stoma.  $\times 17$ .

Fig. 19. Transverse section showing structure of foliar bundle in the lamina.  $x$ , xylem, five tracheides shown;  $px$ , canal, marking position of primitive tracheide; a fragment of its lignified wall is shown; to the right and left are the other two xylem-canals;  $ph$ , phloem.  $\times 310$ .

Fig. 20. Transverse section (cut just above sporangium) of a very young foliar bundle.  $px$ , the primitive tracheide, from which the central canal will be formed; this is at present the only lignified element;  $pph$ , protophloem.  $\times$  about 430.

Fig. 21. Transverse section from a partly developed leaf, showing a persistent transverse wall of the protoxylem, forming a perforated diaphragm across the canal.  $\times 680$ .

Fig. 22. Foliar bundle of lamina, in radial section.  $x$ , a tracheide of the xylem;  $px$ , protoxylem, now forming a canal, crossed by a persistent transverse wall;  $ph$ , phloem.  $\times 296$ .

Fig. 23. Corner of lamina in transverse section, showing a fibrous strand.  $\times 280$ .

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Fig. 24. Callose sieve-plate, shown in longitudinal section of a sieve-tube in the foliar bundle.  $\times 1650$ .

Fig. 25. Sieve-tubes from foliar bundle, showing sieve-areas on the lateral walls.  $\times 720$ .

Fig. 26. Diagrammatic radial section of the base of a sporophyll. *lg*, ligule; *lab*, labium; *i*, velum; *tr*, trabeculae in sporangium; *x*, xylem; *ph*, phloem of the foliar bundle; *t*, subligular tracheal sheath.

Fig. 27. Radial section of rather young leaf, showing base of ligule, *lg*; *t*, tracheal sheath, seen on either side of the ligular base.  $\times 160$ .

Fig. 28. Spinose leaf-base, seen obliquely from above, and from the dorsal side.  $\times 8$ .

Fig. 29. Whole plant, seen obliquely from beneath, after removing the roots and spinose leaf-bases; the three furrows and the living sporophylls are shown.  $\times \frac{3}{2}$ .

Fig. 30. Root, in somewhat diagrammatic transverse section. *x*, xylem; *px*, protoxylem; *ph*, phloem; *c*, cortex.  $\times 68$ .

Fig. 31. Central part of the root, in transverse section, showing stele and intercellular cavity. *end*, endodermis; *x*, xylem; *ph*, phloem.  $\times 320$ .

Fig. 32. Young stele of root, in transverse section. *px*, protoxylem, the only element as yet lignified; *ph*, phloem, adjacent to endodermis.  $\times 580$ .

Fig. 33. Young root-trace, close to stele of stem, in transverse section. *px*, protoxylem, two tracheides lignified at this stage; *ph*, phloem in course of development.  $\times$  about 400.

Fig. 34. Root-stele, in transverse section, at the base of a dichotomy. *x*, xylem, widely expanded laterally; *ph*, phloem.  $\times$  about 400.

# Comparative Anatomy of the Hymenophyllaceae, Schizaeaceae, and Gleicheniaceae.

## I. On the Anatomy of the Hymenophyllaceae<sup>1</sup>.

BY

L. A. BOODLE, F.L.S.

—♦—  
With Plates XXV, XXVI, and XXVII.  
—♦—

IN a note published last December<sup>2</sup> some types of structure found in the natural orders Schizaeaceae, Gleicheniaceae, and Hymenophyllaceae were shortly compared. The present paper contains a more detailed description of the structure of the Hymenophyllaceae, the anatomy of the other two orders, and a general comparison of the three, being reserved for a future paper.

Among the works hitherto published on the Hymenophyllaceae, Prantl's monograph<sup>3</sup> gives a large amount of information on the structure of the leaf and petiole, but his description of the stem is less complete, and needs supplementing. The veins of the leaf and the pseudo-veins (*Scheinerven*) have been fully described by Prantl and other authors<sup>4</sup>;

<sup>1</sup> From the Jodrell Laboratory, Royal Gardens, Kew.

<sup>2</sup> Boodle, *Annals of Botany*, vol. xiii, 1899, p. 624.

<sup>3</sup> Prantl, *Unters. z. Morph. d. Gefässkrypt.*: I. Hymenophyllaceen, 1875.

<sup>4</sup> Mettenius, *Ueber die Hymenophyllaceen*, *Abh. d. k. Sächs. Ges. d. Wiss.*, VII, 1864; Prantl, *l. c.*, &c.



the present paper will therefore be chiefly confined to the stem and petiole.

In the nomenclature of the species to be described Hooker and Baker's Synopsis Filicum is followed, and to save repetition the author of a species is generally quoted only the first time the species is mentioned.

The Hymenophyllaceae include, according to Hooker and Baker<sup>1</sup>, the three genera *Loxsoma*, Br., *Hymenophyllum*, Linn., and *Trichomanes*, Smith. Of these, *Loxsoma* is now excluded from the order by different authors<sup>2</sup>. *Hymenophyllum* and *Trichomanes* have been divided into a number of genera by Presl<sup>3</sup>, Prantl<sup>4</sup> and others, on rather small characters, probably distinguishing sub-genera rather than genera, when valid. Hence regarding *Loxsoma* as excluded, the natural order is here taken as comprising only the two genera *Hymenophyllum* and *Trichomanes*.

In the description of structure, *Hymenophyllum* will be taken first, and the observations arranged in sections dealing with stem, petiole and node respectively.

#### HYMENOPHYLLUM, stem.

The stem in this genus is a creeping rhizome rooted on the lower side, and bearing leaves on the upper side, both roots and leaves being distichous<sup>5</sup>. It exhibits a considerable range of structure, greater complexity occurring in species with comparatively stout rhizome and large leaves. *Hymenophyllum scabrum*, A. Rich., has these characters. The structure of its mature rhizome is illustrated by Figs. 1 and 4, Plate XXV. Fig. 1 is a diagram of the entire transverse section, in which the parts are distinguished as follows:—the outer cortex is left blank, the inner sclerenchymatous cortex (*sc*) is shaded, and the phloem (*ph*) represented by a ring of broken lines; the space between the

<sup>1</sup> Hooker and Baker, Synopsis Filicum, 1874.

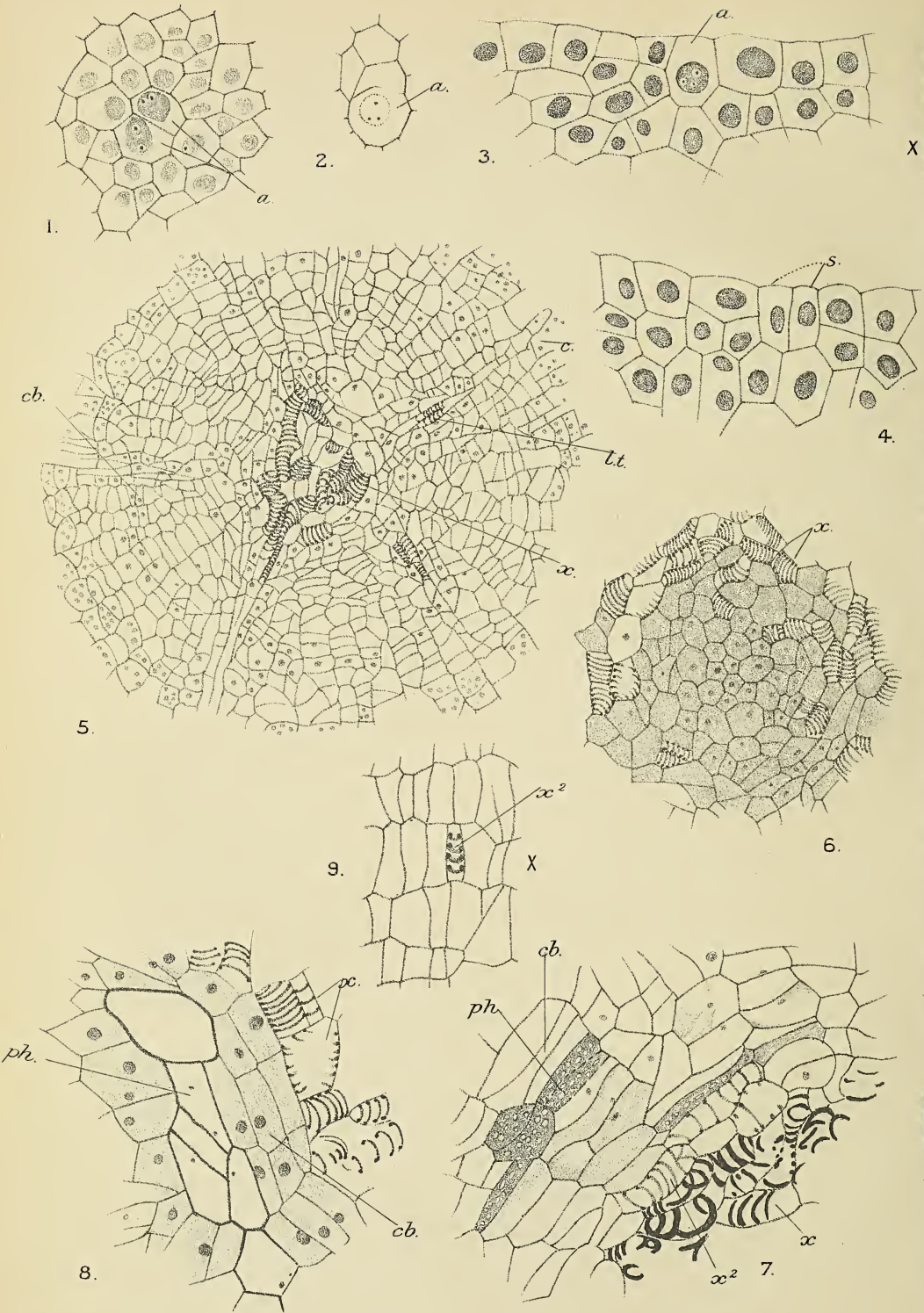
<sup>2</sup> e. g. Christ, Die Farnkräuter der Erde.

<sup>3</sup> Presl, Hymenophyllaceae, 1843.

<sup>4</sup> Prantl, l. c.

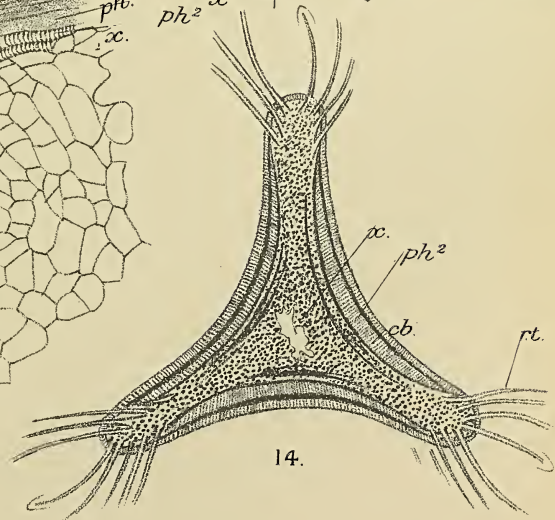
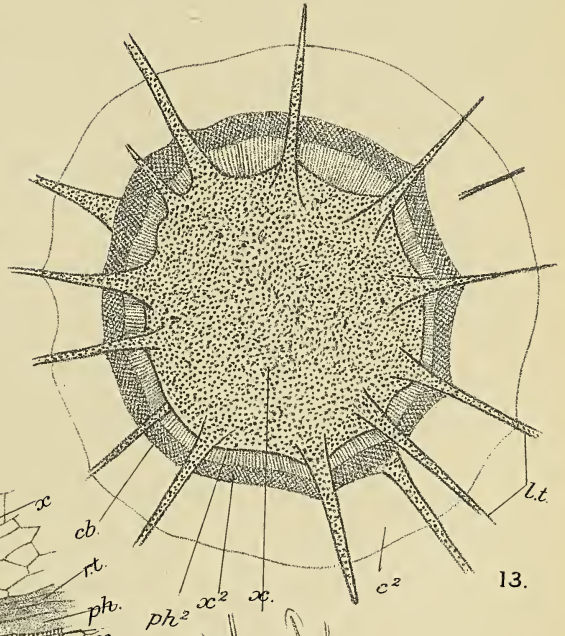
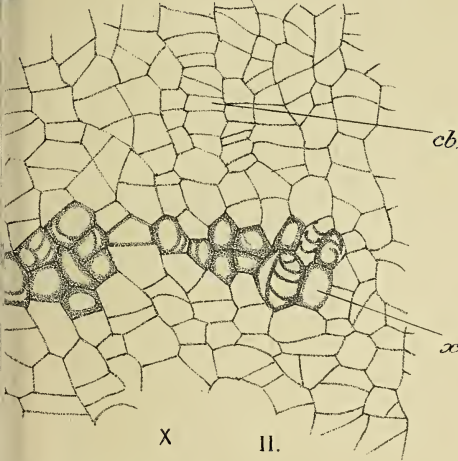
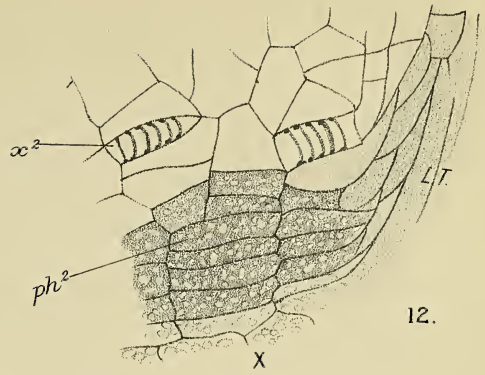
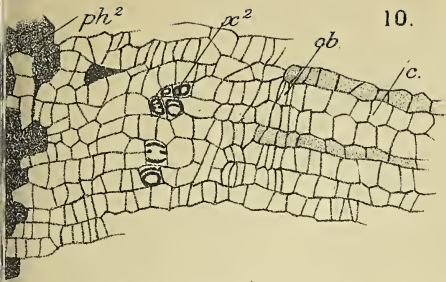
<sup>5</sup> Prantl, l. c., p. 25.



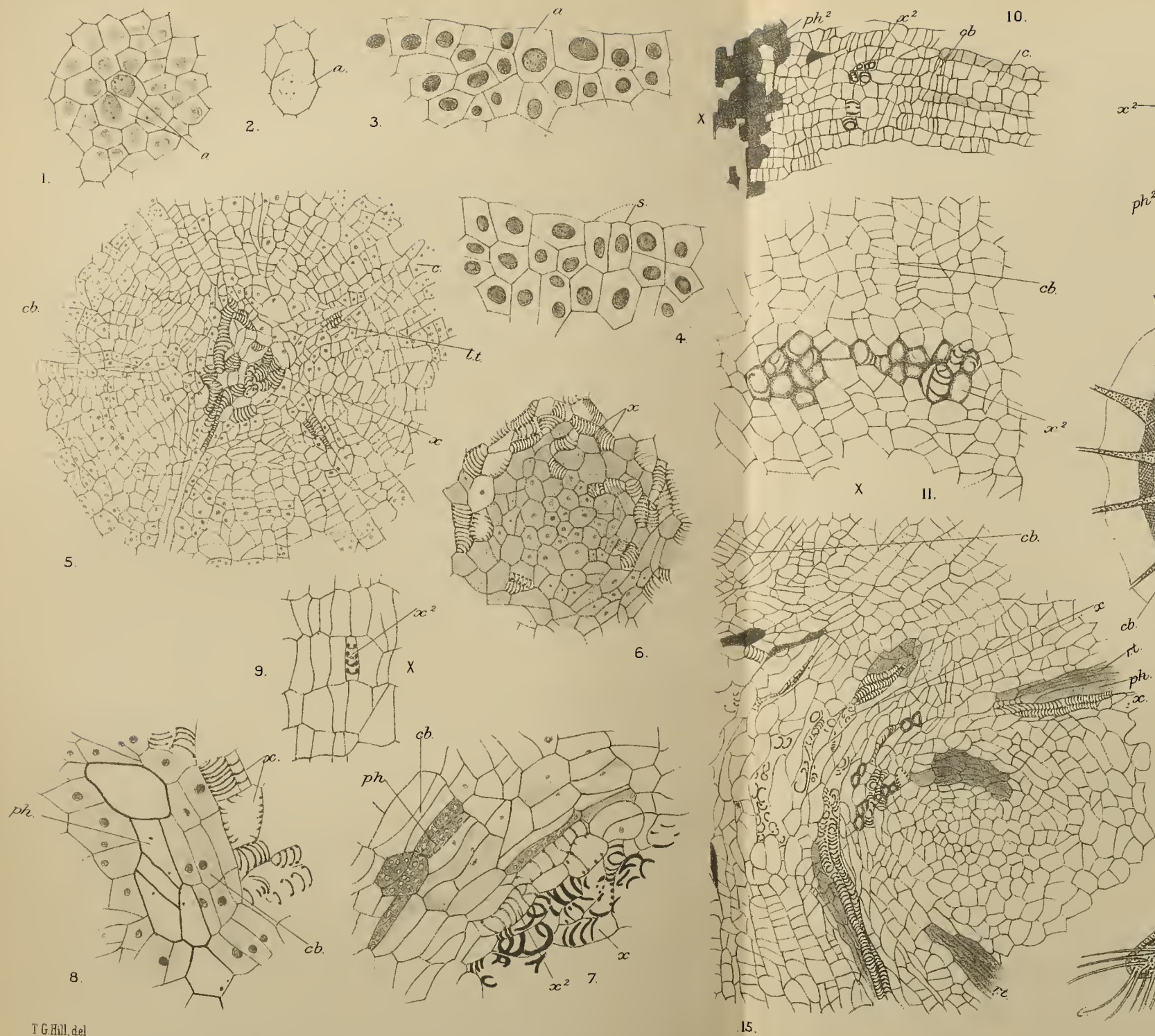


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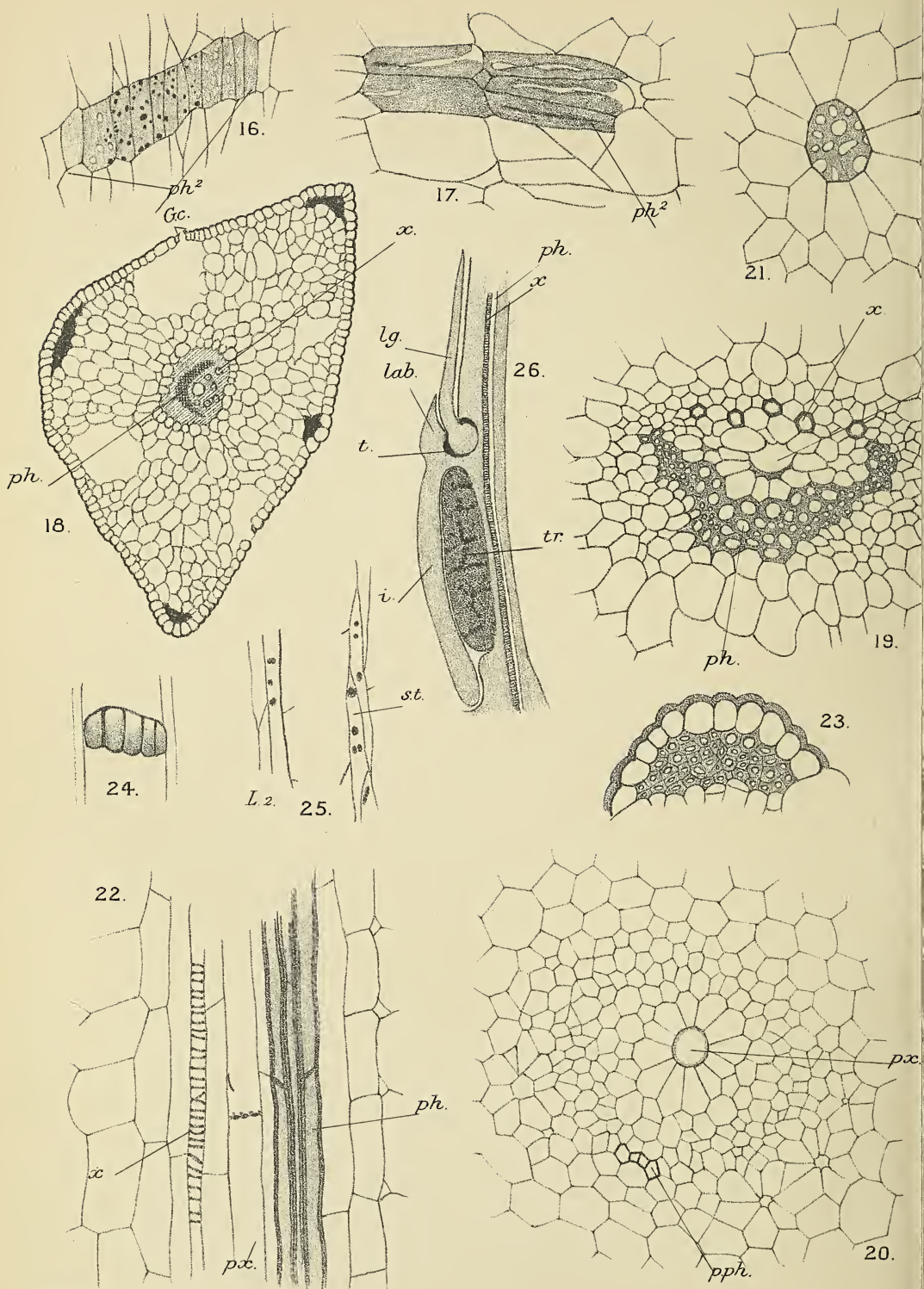
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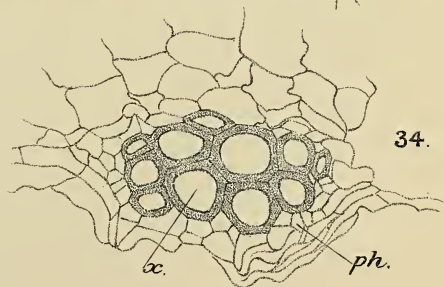
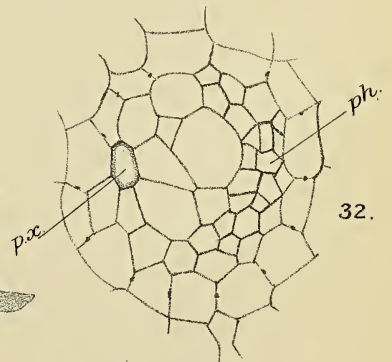
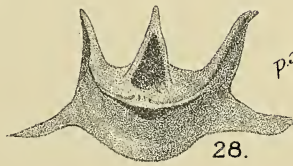
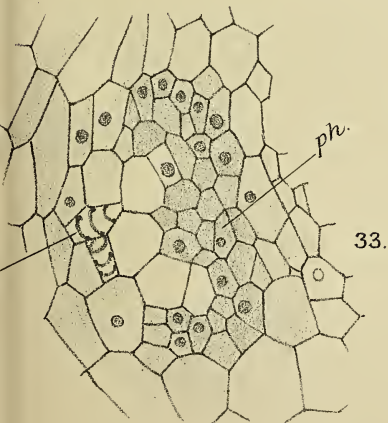
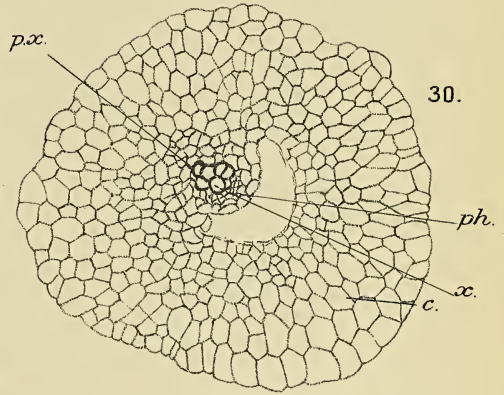
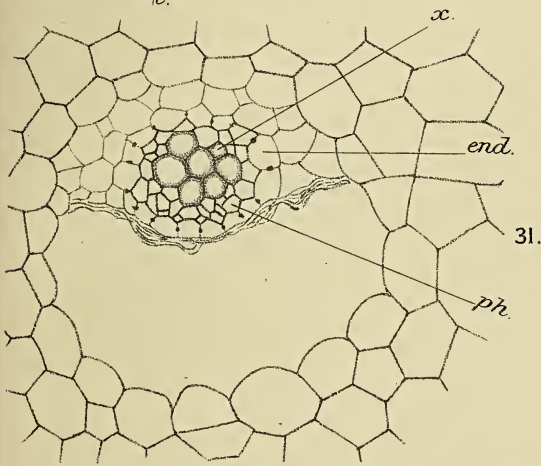
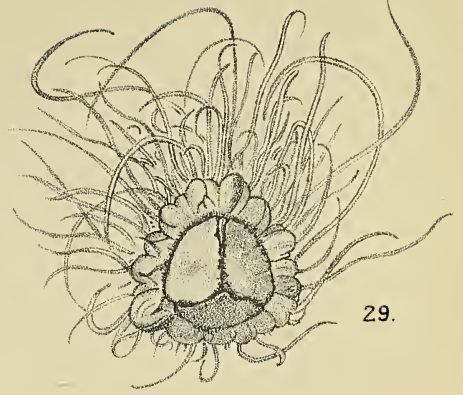




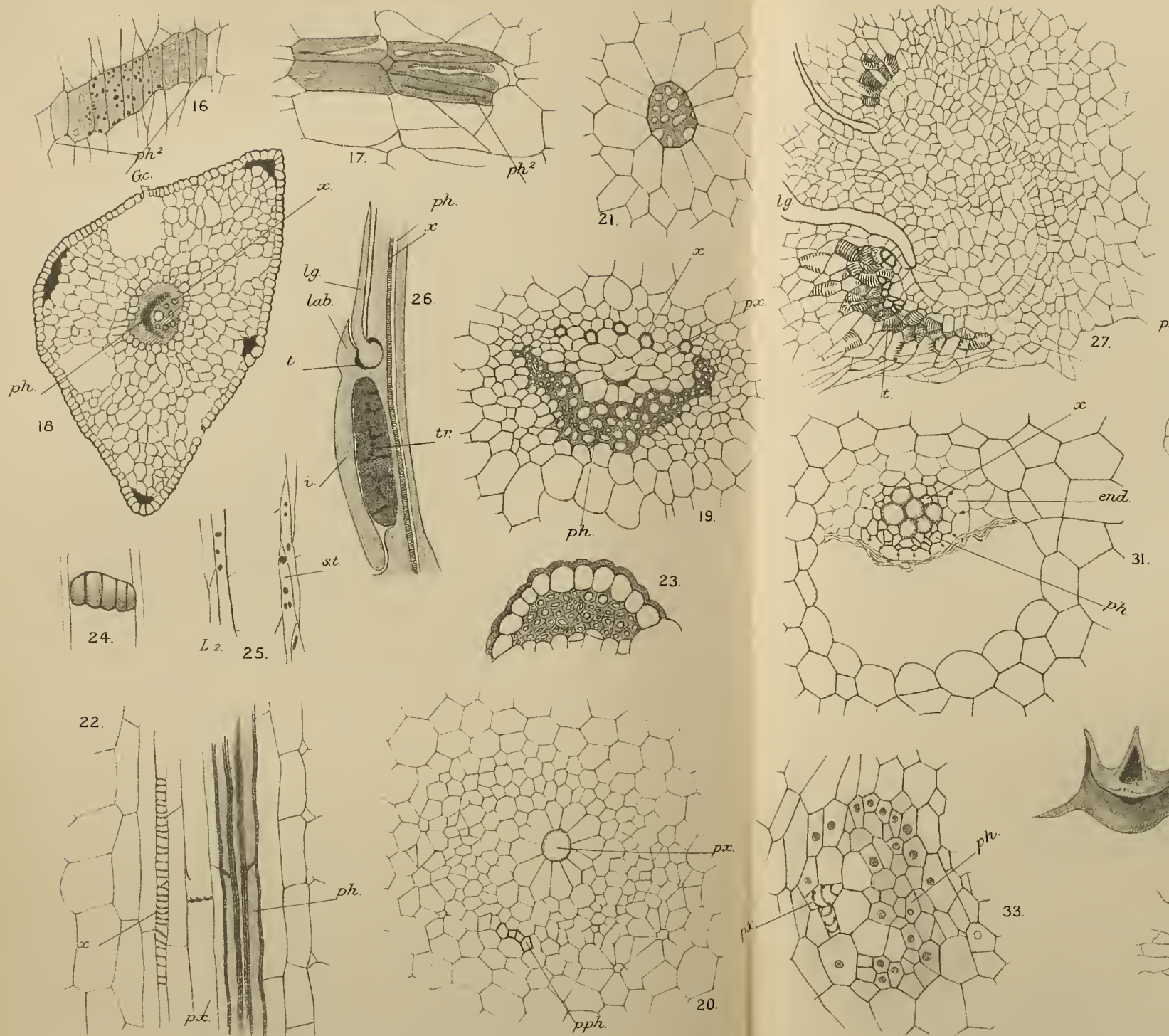


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phloem and the endodermis (*e*) is the pericycle, which is of considerable thickness, usually three or four layers of cells; the conjunctive parenchyma is left blank, and the metaxylem is distinguished by being divided in a brick-like manner; the protoxylem (*px*) is represented by a group of dots. The metaxylem takes the form of an upper xylem-band (*u*), and a lower xylem-band (*l*).

A drawing of the stele of the same rhizome is seen in Fig. 4. The phloem (*ph*) forms a continuous ring<sup>1</sup>, which is thicker on the upper side of the stele than on the lower. The protophloem is at the periphery of this, and is recognized at several points by its small, often flattened elements. The phloem varies in different places from one to five elements in thickness. It consists largely of sieve-tubes, or elements resembling sieve-tubes. Their walls are rather thick and stain deeply with haematoxylin. In longitudinal section these elements are seen to be much elongated, with no evident contents, and with abundant pits on their longitudinal walls. From these characters, and from their arrangement and time of differentiation in the stele, there seems little doubt that they have the function of sieve-tubes, though unfortunately the presence of perforations and of callus has not so far been determined<sup>2</sup>. Though of smaller size, these elements in general characters resemble the sieve-tubes of the root of *Ophioglossum vulgatum*, in which Poirault<sup>3</sup> describes true sieve-structure. Some parenchymatous cells occur here and there among the sieve-tubes, and they have thin walls where they abut on one another, but they have not been distinguished by that character in the drawing.

Conjunctive parenchyma, one or more cells in thickness, separates the phloem from the xylem. The metaxylem consists of two bands of tracheides. Of these the upper (*u*) is more massive, and contains larger tracheides than the

<sup>1</sup> It is interrupted in the neighbourhood of roots.

<sup>2</sup> In the description of the leaf, Prantl (l. c., p. 17) states that sieve-tubes or similar elements could not be found in any plant in the whole order.

<sup>3</sup> Poirault, *Recherches sur les Cryp. Vasc.*, Ann. des Sci. Nat., 7<sup>e</sup> sér., t. xviii, 1893, p. 140.

lower (*l*). Between the two xylem-bands is a zone of conjunctive parenchyma, in which is embedded the protoxylem-group (*px*). The latter is sometimes free, and sometimes abuts on the lower xylem-band. A few tracheides (*r*) form a prominence at the left-hand end of the lower band; they have come in recently from a root. The two rows of roots are inserted at points about radially opposite to the respective ends of the lower band. The protoxylem-elements are spiral and annular; the metaxylem consists of scalariform tracheides. The xylem-bands lie in horizontal planes.

The term 'metaxylem,' when used in the present paper, is applied to all the xylem in a stele or bundle other than protoxylem. The word metaxylem is used by Poirault<sup>1</sup> for tracheides, which are formed on the flanks of the diarch plate of the roots of several species of Ferns, and have a different direction of development from the rest of the xylem-plate. The xylem of these roots is therefore composed, according to Poirault, of protoxylem, deutoxylem (of Van Tieghem) and metaxylem. As, however, there does not appear to be any fundamental difference between the elements of the two latter, and as 'metaxylem' has come into use for primary xylem excluding the protoxylem, the latter terminology is adopted here.

Figs. 2 and 3 illustrate developmental stages of the rhizome of *H. scabrum*. Fig. 2 shows the protophloem (*pph*) differentiated in contact with the pericycle; the small cells succeeding them inwards are the young elements of the remainder of the phloem. The protoxylem (*px*) is seen as a small group of tracheides near the middle of the stele, while the group of four tracheides (*r*) will afterwards form one end of the lower xylem-band. They owe their early differentiation to the presence of a young root; they begin to differentiate later than the true protoxylem. The pericycle is included for a short distance on one side of the drawing, it is two or three cells in thickness, and is limited on the outer side by the endo-

<sup>1</sup> Poirault, Recherches sur les Cryptogames Vasculaires, Ann. des Sci. Nat., Bot., 7<sup>e</sup> sér., t. xviii, p. 141.



dermis (*e*), and on the inner side by the protophloem. The layer of cells with cuticularized bands in its radial walls will here be spoken of in all cases as endodermis<sup>1</sup>, and the layers of cells between it and the phloem as pericycle. These latter layers, according to Van Tieghem, are partly cortical, partly stelar in origin, in *Hymenophyllum*, the outermost layer being formed of sister-cells of the plicated layer (Strasburger's endodermis) which is cortical, the inner layer or layers being stelar and therefore true pericycle<sup>2</sup>. The arrangement of the radial walls tends to confirm the first point, but it appears doubtful whether the inner layers may not also be cortical in origin.

In small rhizomes, such as that of *H. tunbridgense*, Smith (Fig. 16), there is only one layer of cells between endodermis and cortex, so that Strasburger's term 'phloeoterma'<sup>3</sup> might be applied to it, as it appears to represent sister-cells of the endodermis, which is cortical. It seems, however, convenient to remove the morphological meaning from the word pericycle, as Strasburger has done in the case of 'endodermis,' and to use it for any cells between endodermis (of Strasburger) and phloem. Then when the development has been cleared up, one can speak of 'stelar' or 'cortical pericycle.'

In Fig. 3, which is a later stage of the rhizome, the phloem (*ph*) is about fully formed, the protoxylem (*px*) abuts on the lower xylem-band (*l*), which is now complete, while the differentiation of the upper xylem-band has just begun, only two of its tracheides (*u*) having so far been formed. It is thus seen that the lower xylem-band may be fully formed, when the upper band has hardly begun to differentiate. The time of formation of the latter appears to depend on the proximity of leaf-traces. A rhizome of the same age as the above, if there had happened to be a young leaf just in front, would

<sup>1</sup> Strasburger (Ueber Bau u. Verricht. d. Leitungsbahnen, p. 484) uses the word in this way.

<sup>2</sup> Van Tieghem, Sur le dédoublement de l'endoderme, Journ. de Botan., II, 1888, p. 404.

<sup>3</sup> Strasburger, l. c., p. 484.

probably have shown the upper band in a much more advanced stage of development. Fig. 3 only requires the completion of the upper band to produce the mature structure of Fig. 4.

In *H. scabrum* the upper xylem-band is usually separated at both ends from the lower by parenchyma, but this is not always the case. The early differentiation of the lower xylem-band, and the attachment of roots to it, suggest that it is at first important for conduction of water to the growing apex of the rhizome.

*Hymenophyllum demissum*, Swartz, var. *nitens* (Hort.), also has a fair-sized rhizome, the structure of which is very similar to that of *H. scabrum*. Fig. 5 is a diagram of a transverse section. Here the outer cortex is sclerenchymatous as well as the inner, so that the sclerenchyma (*sc*) extends from epidermis to endodermis. The chief features of the stele which differ from *H. scabrum* are that the upper and lower xylem-bands (*u*, *l*) are frequently fused at both ends, and that the protoxylem (*px*) is usually not a compact group, but is spread out as a horizontal band in the conjunctive parenchyma. The xylem, phloem, &c. are lettered as in the previous diagram (Fig. 1). The xylem of a root-stele (*r*) is seen on its way in to join the xylem of the rhizome.

In *H. dilatatum*, Swartz, var. *Forsterianum* (Hort.), the structure of the rhizome is of much the same type as *H. demissum*, as is seen in the stele on the right in Fig. 9. The upper and lower bands are here more frequently free, but the protoxylem is usually spread out as a row of tracheides in the central parenchyma, as in *H. demissum*.

The examples so far described have a rhizome, which is comparatively stout for the genus. Species with more slender rhizomes have a simpler type of structure, and some of them will now be described.

*H. sericeum*, Swartz (Plate XXVI, Fig. 13), has a fair-sized mass of metaxylem (*x*) with either one or two protoxylem-groups attached to its lower side. The section figured has two protoxylems (*px*). The xylem shows no sign of the differentiation into two bands, as seen in the previous types.

The phloem (*ph*) forms a continuous ring surrounding the xylem. The rhizome of *H. fucoides*, Swartz (Fig. 14), has a considerably smaller group of metaxylem-elements, with one protoxylem-group attached to its lower side. The group of small tracheides attached to the right-hand end of the metaxylem is derived from a root. The mass of xylem is still smaller in the rhizome of *H. tunbridgense*, Smith (Fig. 16). (This is seen by comparing Figs. 16 and 14, and allowing for the difference of magnification, Fig. 16 being about one and a half times the scale of Fig. 14.) The pericycle is one cell in thickness. The protoxylem is in contact with the metaxylem, and is on the lower side. The stele has a phloem ring, which may be called continuous, but which tends to have larger elements on the upper side than on the lower, and is more liable to interruptions on the lower side. The xylem is monarch, with the protoxylem on the lower side. If the phloem were not developed on the lower side, the result would be a collateral bundle. To signify this, it is convenient to employ a special term for the type of structure found in *H. tunbridgense*, and the term *sub-collateral* is suggested.

A considerable number of species of *Hymenophyllum* have rhizomes belonging to this type; e.g. besides those just mentioned, the following:—*H. polyanthos*, Swartz, *H. ciliatum*, Swartz, *H. Smithii*, Hook, *H. javanicum*, Spreng. Other species have a similar xylem-mass, and are probably of the same type, but the material was not sufficiently well preserved in the following cases to show whether the phloem surrounded the xylem or was interrupted on the lower side:—*H. denticulatum*, Swartz, *H. Treubii*, Raciborski, *H. fuscum*, Bosch, *H. rarum*, R. Br.

In species with a sub-collateral stele, the tracheides from a root are inserted laterally on the metaxylem, that is in about the same region with regard to the outline of the entire xylem, as in the case of the species with two xylem-bands, e.g. *H. scabrum*.

The two-banded stele of *H. scabrum* may be taken as



typical for species with a comparatively stout rhizome, and the sub-collateral type of *H. tunbridgense* for species with very delicate rhizome.

The rhizomes of large and small specimens of the same species were examined in order to see what differences in structure they would show. It seemed likely that some signs of transition between the two-banded and the sub-collateral types might occur. And this was found to be the case. The size of the stele in the rhizome varies considerably within the limits of a species. As one would expect, a robust plant usually differs from a meagre plant in having a stouter stele, comprising a larger number of tracheides, &c.; the tracheides tending to larger diameter in the larger plant. This difference in several species did not in any way affect the *type* of structure; but the following case is interesting as showing the way in which reduction may sometimes take place, and may have occurred in a phylogenetic series. Fig. 18 is a drawing of the stele (xylem and parenchyma only included) of a large plant of *Hymenophyllum cruentum*, Cav. If this is compared with Fig. 19, which represents the stele of a smaller plant, the following points will be noticed:—the xylem and parenchyma form a much larger mass in the larger plant (Fig. 18)<sup>1</sup>, the tracheides are slightly more numerous in this plant, and are decidedly larger. But the important feature is that in the larger plant there are two groups of tracheides (*l, l*), three on one side and four on the other, belonging to the lower xylem-band, while in the other plant (Fig. 19) either the lower band is entirely absent, or perhaps the two lowest tracheides on one side and one on the other may represent a remnant of the lower band. In Fig. 18 the formation of about four more tracheides would complete the lower band, and the structure would then be of the same type as in *H. scabrum*. In Fig. 19, on the other hand, the xylem arch is more flattened, and a further flattening, or a massing of the metaxylem-elements, would produce the sub-collateral type. This elucidates the manner in which the sub-collateral

<sup>1</sup> The two figures have the same magnification.

structure of the small species of *Hymenophyllum* may have been derived from the more complex type of structure with two xylem-bands, as seen in the larger species. Thus, to state the case more fully, a reduction in requirements of conducting tissue may very possibly have led to a change in structure of the following kind:—Starting with the structure of *H. scabrum*, for instance, by the abortion of part of the lower xylem-band the structure of *H. cruentum* (Fig. 18) would be reached; by the total abortion of the lower band the structure would be reduced to a curved upper band with the protoxylem separated from it by parenchyma: such a type is found in *H. lineare*, Swartz, and apparently also in small plants of *H. cruentum*; finally aggregation of the elements of the upper band, and shifting of the protoxylem into contact with it, would produce the sub-collateral type of *H. tumbridgensis*. Suppression of the phloem on the lower side of the stele would convert the last type into a truly collateral structure. This has not been found so far in any species of *Hymenophyllum*, but it will be described below in the genus *Trichomanes*. Though suppression of the lower phloem does not appear to take place in *Hymenophyllum*, the upper phloem is often much better developed than the lower (see Fig. 14), and in more delicate rhizomes the phloem is often interrupted at one or more points on the lower side (see Figs. 16 and 19), thus showing a tendency to suppression on that side.

The question of the likelihood of the smaller species being reduced, rather than primitive, will be discussed below. Another interesting case of diversity of structure in different specimens of a species was seen in *H. dilatatum*. A large specimen from New Zealand had an oblong stele in its rhizome, in structure like that of the var. *Forsterianum* (see Fig. 9, on the right), while a small plant from Tahiti had a much smaller rounded stele, with a small nearly central protoxylem-group, accompanied by only a little conjunctive parenchyma, and surrounded by the metaxylem, which formed a fairly compact mass of tracheides, not clearly separable into

upper and lower bands <sup>1</sup>. This structure, regarded as a reduction from the type of stele seen in large plants of the species, shows a kind of reduction differing from the cases given above, and consisting in compacting of the xylem and lessening of the central parenchyma. As the protoxylem remains central, there is no tendency towards the sub-collateral type.

A specimen from Java <sup>2</sup>, had a structure very different at first sight from that of the specimens just described. It is shown (xylem only) in Fig. 21. There appears to be only one xylem-band, but as the protoxylem (*px*) is immersed in the metaxylem, it does not belong to the sub-collateral type, but the tracheides below the protoxylem elements represent part of the lower band. It is really similar to the specimen from Tahiti, the xylem being compacted, and the reduction of intraxylar parenchyma having gone still further; the chief difference being that the xylem forms a band instead of a circular mass. *H. flabellatum*, Labill., has in its rhizome the same type of structure as exhibited by the specimen of *H. dilatatum* from Tahiti. Several different-sized plants of *H. demissum* were examined, and presented differences in the size of the stele, but no departure from the type of *H. demissum*, var. *nitens*, except in the case of a small sterile specimen from Otago <sup>3</sup>, where the lower band was missing, and the structure was therefore as in *H. lineare*.

It must be pointed out that equally marked differences in structure might occur in others of the species described above. Thus in *H. lineare*, where only one specimen was examined, a larger plant might possibly have had a lower xylem-band, or a smaller plant might have shown typical sub-collateral structure.

<sup>1</sup> This shows an approach on a small scale to the structure of *Trichomanes radicans*, as will be seen by referring to the description of the latter.

<sup>2</sup> Collected by Raciborski and identified as *H. Junghuhnii*, Bosch., which is regarded by Hooker and Baker (Synopsis Filicum, p. 62) as a synonym of *H. dilatatum*.

<sup>3</sup> Mr. J. G. Baker kindly gave me his opinion that this specimen was correctly named.



One may summarize the structural characters of the stele thus:—

1. The phloem forms a ring round the xylem.
2. In species with large rhizomes the metaxylem forms a ring enclosing parenchyma and protoxylem; the metaxylem often has the form of two bands.
3. In species with small rhizomes the metaxylem forms a small band or mass, and the protoxylem is peripheral to it on the lower side.
4. Signs of transition between 2 and 3 occur.

#### HYMENOPHYLLUM, roots.

In *Hymenophyllum* the roots are described as being mostly diarch. *H. demissum*, var. *nitens*, may be mentioned as having a typical diarch root, while the root-stele of *H. dilatatum*, var. *Fosterianum*, is much smaller, and appears to be monarch; the root-stele of *H. scabrum* in passing through the cortex of the rhizome also appears to be monarch. In this genus and in *Trichomanes* the root structure does not appear to be of any great interest, apart from the fact that the number of protoxylem-groups differs according to the stoutness of the stele<sup>1</sup>. The higher numbers of protoxylems are found only in *Trichomanes*. The largest number found by Russow<sup>2</sup> was eight, and by Prantl<sup>3</sup> nine. Russow<sup>4</sup> thought that diarch roots were restricted to *Hymenophyllum*, monarch and triarch up to octarch roots being found in *Trichomanes*, but Prantl<sup>5</sup> found that this rule did not hold good. Where monarch roots occur in the Hymenophyllaceae they are probably so by reduction, because the primary root of *Trichomanes alatum* is diarch, as proved by Leclerc du Sablon<sup>6</sup>.

<sup>1</sup> Prantl, l. c., p. 31.

<sup>2</sup> Russow, Vergleich. Unters. d. Leitbündel-Krypt., Mém. de l'Acad. Imp. d. St. Pétersb., VII, 19, 1872, p. 95.

<sup>3</sup> Prantl, l. c., p. 31.

<sup>4</sup> Russow, l. c., p. 95, note.

<sup>5</sup> Prantl, l. c., p. 31.

<sup>6</sup> Leclerc du Sablon, Ann. des Sci. Nat., 7<sup>e</sup> sér., t. xi, p. 111.

## HYMENOPHYLLUM, petiole.

The structure of the petiole of *Hymenophyllum dilatatum*, var. *Forsterianum*, is shown diagrammatically in Plate XXV, Fig. 6. The pericycle is thick as in the rhizome, and the phloem is missing on the upper side of the petiole, which is the lower side in the diagram<sup>1</sup>. The xylem has the form of an arch with its ends turned inwards. Fig. 7 is a drawing of the petiolar bundle of the same plant. A protoxylem-group ( $px^1$ ) is seen at the end of each of the two arms of the xylem, and one of the arms is separated, in this section, from the rest of the metaxylem by parenchyma. Figs. 6 and 7 represent the structure of the petiole as one finds it for a considerable part of its length. A marked difference is seen, however, for some little distance above the axillary branch<sup>2</sup>. The section shown in Fig. 8 was cut through this region. Comparing this with Fig. 6, the difference observed is that the phloem has become continuous, the xylem has also formed a closed ring, and the two protoxylem-groups have become, at any rate partly, internal to the xylem-ring. The type of structure of this region of the petiole is therefore practically stem-like. In the disposition of its elements it is scarcely different from the stem of the same species (cf. Fig. 9, right side).

The petiole of *H. cruentum* (Plate XXVI, Fig. 20) is of the same type as *H. dilatatum*, var. *Forsterianum*, but the bundle is much smaller. The protoxylem ( $px$ ) is at the ends of the two curved arms of the xylem, and the phloem is interrupted on the upper side (the lower in the drawing). At *s.t.* a sieve-tube lies in contact with a tracheide.

Most species of *Hymenophyllum*, which have a sub-collateral structure in their rhizome, have a collateral structure in their petioles, the arrangement of elements in the two being very similar, the difference being that the phloem is continuous in the rhizome, but interrupted in the petiole. Fig. 17 repre-

<sup>1</sup> All the petioles are drawn orientated in this way.

<sup>2</sup> The occurrence of axillary branches will be described below.

sents the petiolar bundle of *H. tunbridgense*, and should be compared with the rhizome of the same species (Fig. 16). Another case of a sieve-tube in contact with a tracheide occurs in Fig. 17. Figs. 15 and 14 show the structure of the petiole and rhizome respectively of *H. fucoides*; the same difference is presented as in *H. tunbridgense*. In *H. polyanthos* and *H. Smithii* the petiolar bundle is collateral, with a xylem of the form found in the rhizome, while in *H. javanicum* and other species it could not be determined whether the phloem was interrupted, the material not being well enough preserved. *H. sericeum* and *H. ciliatum* differed from the other plants of this series in not having typical collateral structure in their petioles. In *H. sericeum* the xylem is shaped like the letter Y, with protoxylem forming the ends of the two arms, and in *H. ciliatum* the xylem is very small, but has the form of a curved arch with the ends turned inward, and with protoxylem at the two extremities; in this species two or three scattered sieve-tubes occurred on the upper side.

#### HYMENOPHYLLUM, nodal region.

Axillary branches occur very generally in the *Hymenophyllaceae*<sup>1</sup>, at many nodes, however, the rudiment of the axillary branch may remain undeveloped. The stele of the axillary branch is inserted on the leaf-trace. Figs. 9-12 are drawings of transverse sections of the node of *H. dilatatum*, var. *Forsterianum*, which show the behaviour of the leaf-trace and axillary branch. In Fig. 9, Plate XXV, the stele of the rhizome (*s*) is seen on the right, cut transversely, and on the left the leaf-trace (*l.t.*) is fusing with the stele of the axillary branch, both of them being in oblique section. In Fig. 10 the large stele on the right belongs to the rhizome, the small one on the left is the leaf-trace, i.e. the product of fusion of the leaf-trace and stele of the branch. The phloem of the leaf-trace (*ph*<sup>1</sup>) may be recognized by the small size of its elements as compared with the pericyclic cells, and forms

<sup>1</sup> Prantl, l. c., p. 25.



a practically continuous ring ; the xylem also forms a ring, and shows a differentiation similar to the upper and lower bands of the rhizome, so that a slight shifting of the protoxylem would make the structure identical with that of the rhizome. Close above the axillary branch, as has been described, the petiolar bundle is still more like the stele of the rhizome. Fig. 11 shows the further fusion of the two steles of the previous figure. Here the right and left of the last figure are reversed. The metaxylem ( $x^1$ ) of the leaf-trace has become attached to the upper band of the rhizome ;  $px^1$  is the protoxylem of the leaf-trace. Fig. 12, Plate XXVI (orientated as in Fig. 10), is a further stage, in which the metaxylem of the leaf-trace and the upper xylem-band of the rhizome form a single curved arc, while the protoxylem of the leaf-trace ( $px^1$ ) is turning in towards the central parenchyma. It afterwards passes further in and joins the other protoxylem-elements ( $px$ ) already present in the central parenchyma of the stele, which, no doubt, are continuous with the protoxylem of other leaf-traces. In sub-collateral species the leaf-trace and the stele come into lateral contact, and fuse. The two protoxylems may remain distinct for a short distance.

The fact that the basal region of the petiole approaches stem-structure, may be due to physiological requirements. It has to connect two similarly constructed organs, the rhizome and axillary branch, so that a closed xylem-ring and continuous phloem, resembling their structure, is probably well suited for connecting the vascular elements of the two.

As to the water supply of the leaf in a species like the above : the protoxylem-elements appear to be all in contact with one another at some points, and some of them with the lower xylem-band as seen in Fig. 12, so conduction may go on from the roots through the lower xylem-band to the protoxylem in the rhizome, and the protoxylem in the leaf-trace. The rest of the water must pass from the lower band to the upper (either at occasional points of union, or across the central parenchyma) and from there to the metaxylem of the leaf-trace. In *H. demissum*, var. *nitens*, where the two bands

are mostly fused, the roots are in direct connexion with both, being attached to the lines of junction of the two.

#### TRICHOMANES.

In the genus *Hymenophyllum*, all the species have a creeping rhizome; but in *Trichomanes*, while many species have a similar habit, others have a short erect or oblique stem. The leaves are arranged distichously on the creeping rhizome, but have a two-fifth phyllotaxis in the case of upright stems, e. g. *T. Prieurii*, Kunze. The description of structure in the genus *Trichomanes* will be arranged under the different species, as it will be more convenient to take rhizome, petiole, and node of one species before going on to the rhizome of the next.

##### *T. reniforme*, Forst.

This species has a creeping rhizome and distichous arrangement of leaves and roots, as in *Hymenophyllum*, but it has a simple leaf, which is not very common in the order. The lamina is reniform in shape, and is unique in being four cells thick.

The structure is of almost the same type as in the larger species of *Hymenophyllum*, so it will be suitable to begin with. The stele of the rhizome is shown in Fig. 22. It has much in common with the type of *H. dilatatum* or *H. scabrum*, but differs in some points of detail. The xylem consists of an upper and lower band; their limits, however, are sometimes not distinct, and the difference in the size of the tracheides is not conspicuous, but the upper xylem sometimes contains distinctly larger tracheides than the lower. Further the central mass of parenchyma contains, in addition to the evident protoxylem-group or groups, several larger tracheides, which must be regarded as forming part of the metaxylem. There are some for instance in Fig. 22, separating the two protoxylem-groups there present. The root-steles are attached to the ends of the lower xylem-band (or to the junction of the two bands) as in *Hymenophyllum*.

Fig. 23 shows a young stage of the rhizome in transverse

section. The drawing includes the phloem (*ph*), and excludes the pericycle. The protoxylem in this section is centrally placed, and the two groups of tracheides (*r*, *r*) are the ends of the lower xylem-band, which develop early in connexion with the roots, as in *Hymenophyllum*. The group on the right is cut near the insertion of a root-stele, as shown by the phloem being curved out into a prominence opposite to it.

In the petiole, at its base, the xylem forms a continuous ring, enclosing central parenchyma, with protoxylem and a little metaxylem included in it, the whole being surrounded by a continuous ring of phloem. Thus the structure in this region is almost identical with that of the rhizome. High up in the petiole a small gap may occur on the upper side in the xylem-ring, and the sieve-tubes become scanty and scattered on the same side. The protoxylem comes to abut on the inner surface of the upper band of xylem (or is embedded in it), and becomes separated into two groups. This is just below the lamina. Some metaxylem then forms a bridge across the central parenchyma, in the median plane of the petiole. The xylem thus resembles two circles pressed together so as to be flattened where they touch. In the very base of the lamina the two circles of xylem split apart, and phloem is formed on the sides where they face one another, so that two perfectly similar concentric bundles are formed. They are the two primary veins of the leaf, and are thus formed by dichotomy of the petiolar bundle. By their repeated forking they produce the venation of the leaf.

The retention of basal petiolar (stem-like) structure up to the top of the petiole seems to be connected with the subsequent division of the bundle by dichotomy. In species of *Hymenophyllum* with a similar structure at the base of the petiole, the xylem-ring opens into an arc, a little higher up, the bundle assuming a form which is probably more suitable for the detachment of bundles for the pinnae right and left. As the dichotomous venation has been regarded as a primitive character<sup>1</sup>, a similarly shaped leaf, namely that of

<sup>1</sup> Prantl, l. c., p. 57.



*Adiantum reniforme*, Linn., was examined, and it was found that there also the first division of the petiolar bundle at the base of the lamina was dichotomous. This fact does not favour the view that dichotomous venation is, of itself, important evidence of a primitive nature.

At the node in *T. reniforme* the leaf-trace approaches the side of the stele of the rhizome, the xylem of each opens at the point of contact, and the two xylem bands of the petiole fuse with the corresponding bands of the rhizome, while the protoxylem of the leaf-trace passes towards the other group of protoxylem in the central parenchyma. The presence of two protoxylems in the rhizome, in Fig. 22, means that the section was cut not far from a node, but Fig. 23 represents a section cut through the region of an internode, which has only one protoxylem.

The fusion of the leaf-trace with the stele is much more like the union of two similar structures than in *Hymenophyllum*.

*T. radicans*, Swartz.

The structure of the rhizome of this species is shown in Fig. 24. The stele has a broad pericycle, as in most other Hymenophyllaceae, the phloem forms a continuous ring, and the xylem consists of a large solid mass of tracheides interspersed with parenchyma, and containing two protoxylem-groups<sup>1</sup> near the centre, each accompanied by a small mass of parenchyma. The parenchyma-groups are easily distinguished in the figure, but the protoxylem-elements are not easy to recognize. The two protoxylem-groups are seen in most sections of the internode, but they fuse into one a little way above the node.

The protoxylem-elements are spiral, and occur in and around each mass of parenchyma. Sections of the young internode prove that they are the first tracheides to develop, and that the first metaxylem differentiated is that immediately surrounding each group of parenchyma and protoxylem. Some small and rather early peripheral tracheides are some-

<sup>1</sup> Two protoxylem-groups are mentioned by Prantl (l. c., p. 27).

times seen. They are connected with a root, and occur on the lower side.

The rhizome bears distichous leaves and roots, but is very stout compared with *T. reniforme* or *Hymenophyllum*, and, as seen in the figure just described, it contains a very much larger stele, but though the amount of xylem is greatly increased, it is really of almost the same type as *T. reniforme*. As in the latter plant, there is internal protoxylem consisting of either one or two groups, according to the distance from the node, and lying in the horizontal plane. *T. reniforme* has several metaxylem elements in the central parenchyma as well as the protoxylem, and in *T. radicans* the central parenchyma is mostly replaced by metaxylem, so as to leave only a small group of parenchyma accompanying each protoxylem. According as one regards the one type or the other as the more primitive, parenchyma must have been phylogenetically replaced by tracheides, or tracheides by parenchyma.

In the petiole the metaxylem has the form of an arch, with its ends turned in, and inclining to a triangular outline. This is seen in Fig. 26, Plate XXVII, which is a diagram made in the same manner as the previous diagrammatic drawings. The protoxylem is not, as might have been expected, restricted to the ends of the two incurved arms of the xylem (which is the position it occupies in the petiole of *Hymenophyllum dilatatum*, Fig. 7), but it is distributed at a few points (represented by dots in the diagram) in the central parenchyma. In this tissue in the mature petiole one recognizes the protoxylem as small and partly collapsed tracheides, some being in contact with the ends of the xylem-arc on their inner surfaces. The phloem is almost continuous, but much scantier on the upper side, where it is interrupted by about one or two parenchymatous cells at the median point. Fig. 27 is a diagram of the structure a short distance above the insertion of the axillary branch. The xylem is continuous, the arms having united<sup>1</sup>, and the central parenchyma contains

<sup>1</sup> This conversion of the xylem-arch into a closed ring at the base of the petiole is mentioned by Prantl (l. c., p. 19).

scattered protoxylem-elements, and, in the case illustrated, one small island and two bridges of metaxylem. The phloem is continuous, but still scanty on the upper side. A drawing of the young stage of the petiole, cut through its lower region, is seen in Fig. 25. The large cells ( $x'$ ) are developing tracheides of the metaxylem arc, and there are four groups of fully developed tracheides ( $px'$ ) representing the protoxylem. The two lower ones will, judging by their position, abut on the arms of the metaxylem. The position of the protoxylem-elements appears to be variable, but, at any rate, several of them lie immersed in the parenchyma. In the mature petiole the partly collapsed protoxylem-elements are not very noticeable, and were overlooked by Prantl in *T. speciosum*, Willd., in which he includes certain forms from Ireland and Madeira falling under *T. radicans* in Hooker and Baker's Synopsis. Prantl describes and figures diagrammatically (l. c., Fig. 51) the protoxylem as occupying the two lateral angles of the triangular xylem.

As in previous cases, the base of the petiole is more stem-like than the upper part, in having a closed ring of xylem with internal protoxylem.

The nodal structure is illustrated diagrammatically by Figs. 28–32. Fig. 28 shows the relative positions of the axillary branch ( $br$ ), the petiole ( $pet$ ) and the main stem in longitudinal section, the vascular tissue being shown lighter than the cortex. The stele of the branch is attached to the leaf-trace just below the point where the latter curves sharply out into the petiole<sup>1</sup>. This is not universally the case, however, as in one node of this species the stele of the branch was attached directly to that of the main stem, a short distance above the attachment of the leaf-trace. The next three diagrams represent transverse sections through the nodal region of the same species. In Fig. 29 the leaf-trace and the steles of the stem and axillary branch are all distinct. Fig. 30 shows leaf-trace and stele of axillary branch fused, but their

<sup>1</sup> This is described by Prantl (l. c.), but his diagram (of another species), Fig. 63, does not make it clear.



protoxylems (represented by dots) still distinct; in Fig. 31 the 'stele,' produced by the union of leaf-trace and stele of axillary branch, is fusing with the stele of the stem, the two phloems having become continuous; and in Fig. 32 the fusion is complete, and internodal structure is attained. In passing down the internode the xylem becomes more rounded, but the protoxylems of the stem and leaf-trace remain distinct, very nearly as far as the next node below, so that a transverse section of an internode usually shows two protoxylems in the stele. In one case the protoxylems were still separate at a distance of 11 mm. below a node, but at 18 mm. below, which was a few mm. above the succeeding node, the protoxylems were practically fused. Thus it is seen that the protoxylem-groups in the stem of this species of *Trichomanes*, as in *Hymenophyllum*, are the direct continuations of those of the leaf-traces. The two protoxylems seen in the internode are connected with the next and next-but-one leaves above. The attachment of the stele of the axillary branch to the leaf-trace also agrees with what is found in *Hymenophyllum*. A comparison of the leaf-trace in Fig. 29 with the petiolar bundle in Fig. 27 shows that after leaving the cortex of the stem its phloem becomes again continuous in the base of the petiole, to be interrupted once more in the upper region of the petiole. The roots are rather stout, and their steles may be attached by a broad base to the xylem on the lower side of the stele.

*T. Pricurii*, Kunze.

This plant differs from all the cases described above, but agrees with some other species of its genus, in that the stem is upright, and the leaf-arrangement is radial. The phylotaxis is two-fifths. The stem has a solid-looking mass of xylem consisting of tracheides mixed with parenchyma in about the same proportion as *T. radicans*, or with less parenchyma. The protoxylems are internal, and as many as three may sometimes be seen in a transverse section. A fair-sized group of sclerenchyma-fibres accompanies the leaf-trace into the stele,

and is found on the inner side of a protoxylem-group, but, in passing downwards, the fibres soon decrease in number until none are left. The protoxylem is then difficult to recognize in transverse sections, as there is only a small amount of parenchyma accompanying it, not a conspicuous group such as occurs in *T. radicans*. The protoxylem tracheides are mostly spiral.

In transverse section the petiolar bundle has the outline of a truncated triangle with the base bulged in. The central region of the bundle is occupied by a large mass of fibres<sup>1</sup>, round which the xylem and phloem form two parallel bands, but are both interrupted at the middle of the upper side. The xylem thus forms a long band in the form of a flat-topped arch with its ends turned in. The protoxylem forms four groups at the four outer angles of the xylem. Besides the centrally placed sclerenchyma, several fibres occur singly or in small groups between xylem and phloem, and also embedded in the xylem. The fibres have small simple pits, and often have cell-contents. They are evidently derived from parenchymatous elements, the central mass of fibres representing the central parenchyma of the petiolar bundle of *T. radicans*, or rather of some form with central parenchyma not containing protoxylem elements. The rest of the fibres found between xylem and phloem, and in the xylem, are derived from the conjunctive parenchyma-cells in these parts, and are just equal to them in size, but differ by their very thick walls. The occurrence of fibres inside a petiolar bundle is not very common among Ferns, but a similar large mass of fibres is found in *Schizaea* and in *Adiantum trapeziforme*, Linn.<sup>2</sup>

Prantl<sup>3</sup> describes the petiole of *T. Prieurii* as having three protoxylems, except just at the base where the dorsal group splits into two. The number of protoxylem-groups depends on the size of the xylem, which varies with the size of the

<sup>1</sup> Mentioned by Mettenius, Russow, Prantl, &c.

<sup>2</sup> Thomae, *Die Blattstiele der Farne*, Pringsheim's Jahrb., XVII, 1886, p. 129. Potonié, *Jahrb. d. k. bot. Gart. Berlin*, II, 1883, p. 242.

<sup>3</sup> Prantl, l. c., p. 22.

leaf. A small, early leaf of a plant had a comparatively small bundle, in which there appeared to be only two protoxylems.

The leaf-trace in the stem has a continuous ring of metaxylem, enclosing a central mass of sclerenchyma, and surrounded by phloem except on the adaxial side. There appear to be three protoxylem-groups lying between the metaxylem and sclerenchyma; one median and anterior, and two lateral. The nodal structure is illustrated by diagrams (Figs. 33-36). In Fig. 33 the stele of the stem is shown at  $x$ ; it contains two masses of sclerenchyma (which are shaded), derived from the next two leaves above. The leaf-trace ( $x'$ ) is seen on its way in through the cortex; the shaded region inside the leaf-trace is the sclerenchyma; and  $a$  is the axillary branch. In Fig. 34 one of the sclerenchyma-masses has disappeared from the stele of the stem, and the stele of the axillary branch has fused with the leaf-trace. In Fig. 35 the second sclerenchyma-group has disappeared from the stele, and in Fig. 36 the leaf-trace is uniting with the stele of the stem, while a new leaf-trace ( $x''$ ) with its axillary branch ( $a$ ) is coming in through the cortex. The xylem-mass of the leaf-trace with the sclerenchyma inside it fuses with the stele of the stem without any great change in arrangement of elements at first, consequently the protoxylem becomes immersed in the xylem of the stele, which is therefore mesarch. Fig. 37 is an enlarged diagram of the stele ( $x$ ) in Fig. 33. The sclerenchyma is shaded, and such protoxylems as could be determined are represented by dots. Connected with the right-hand mass of sclerenchyma are seen the two lateral protoxylems of the petiole, the median one being absent or unrecognizable. On the left only one protoxylem is seen, probably produced by fusion of two lateral groups corresponding to those on the right. *T. Prieurii* is exceptional in containing fibres in its petiole, and it has a two-fifth phyllotaxy, but allowing for the difference this causes in the stele, it does not diverge much from the type of *T. radicans*. There is less parenchyma accompanying the protoxylems, and two or three protoxylems pass in from



the leaf instead of one. Judging by the mature condition it seems that the protoxylem from a leaf may die out in the stem at some distance below its entry into the stele, without fusing with the protoxylem of other leaves, but this could not be proved in the material used.

*T. spicatum*, Hedw.

This is another species with an upright stem and two-fifths arrangement of leaves. The xylem of the stele here consists of a solid mass of tracheides and parenchyma, somewhat as in *T. Prieurii*, but of smaller dimensions, and the tracheides are of nearly uniform size, so that the protoxylem cannot be distinguished in the transverse section of the mature stem. A young stage shows that the first formed tracheides are scattered irregularly. They are not spiral, but are finely scalariform. The petiolar bundle is small, with a ring of metaxylem surrounding a little parenchyma, with a nearly central protoxylem, the phloem being interrupted on the adaxial side. Lower down, the metaxylem next the axis is not formed, so the bundle there contains a band or arc of xylem with the protoxylem on the side towards the stem<sup>1</sup>. The leaf-trace in the stem is similar. Hence the protoxylem is likely to be embedded in the xylem of the stem, and some of the scattered protoxylem-elements in the stem are perhaps continuous with leaf-traces.

*T. Bancroftii*, Hk. and Gr.

This is also a species with an upright stem, and in structure it is very similar to *T. spicatum*, having no spiral protoxylem.

The last three species all have rather thick-walled tracheides. The petiolar bundle has its xylem in the form of a straight band with a protoxylem at each end. The protoxylems do not interrupt the phloem, as stated by Prantl<sup>2</sup>.

<sup>1</sup> This change is the reverse of what occurs in *Hymenophyllum dilatatum*, &c.

<sup>2</sup> Prantl, l. c., p. 21.

Fig. 42 represents some bordered pits of a tracheide. They were drawn on account of their unusual shape. One or two tracheides with similar pits were seen in this species. The other tracheides are of the usual scalariform type as in other species of the genus. The pits illustrated differ from typical structure in their narrowness and in the large size of the border.

*T. heterophyllum*, H.B.K.

This is a species with a creeping rhizome, but it has a stele, which appears to be of the same type as *T. spicatum*, but the tracheides are larger. In the dried material examined the position of the protoxylem could not be determined, but it appeared that no spiral elements were present.

*T. scandens*, Linn.

The stem of this species is a creeping rhizome, and has a large stele. The tracheides are large and separated by a network of parenchyma (Fig. 38), and the protoxylem consists of small spiral tracheides (*px*) which are scattered round the periphery of the xylem. Sections of a young rhizome proved that these were the first tracheides to differentiate. Thus *T. scandens* differs from all the species described above in having a circular mass of xylem, with distinctly peripheral protoxylem.

The petiole has a bundle with a slightly arched xylem-mass. As seen in the young stage (Fig. 39), there are three protoxylems (*px*<sup>1</sup>). The phloem is interrupted on the upper side, and it appears to be just interrupted by the median protoxylem-group. The leaf-trace in the stem probably has peripheral protoxylem on its outer side. At the node some spiral tracheides were seen on the outer side of the leaf-trace, and continued downwards at the periphery of the stele of the stem.

*T. apiifolium*, Presl.

This species has a larger stele, which appears to be of the same type as *T. scandens*.

The petiole has a large bundle, shaped like a squarish arch (Fig. 40). The endodermis, consisting of torn and crushed cells in the dried material, followed the outline of the xylem; the protoxylem formed two groups in the dark tissue separating the incurved tips of the xylem-arch from the uprights of the arch, and there may perhaps have been a third protoxylem in the median region of the arch.

*T. ericoides*, Hedw., &c.

*T. ericoides*, Hedw., *T. alatum*, Swartz, and *T. rigidum*, Swartz, are species with a solid stele in the stem like that of *T. spicatum* or *T. scandens*. *T. alatum* rather resembles *T. spicatum*, but *T. ericoides* and *T. rigidum* have more the characters of *T. scandens* in the size of their tracheides, &c. The position of the protoxylem could unfortunately not be determined, as dried material was used, so it cannot be stated to which type these species belong.

*T. pyxidiferum*, Linn., &c.

Several species of *Trichomanes* show the sub-collateral type of structure in their rhizome; e.g. *T. pyxidiferum*, Linn., where the phloem surrounds the small xylem mass. The xylem may consist of only eight tracheides, and in such cases the protoxylem cannot easily be determined. *T. trichodeum*, Swartz, is similar, and the protoxylem is here clearly peripheral. The petiolar bundle is somewhat similar in structure, but with fewer tracheides, and with diarch arrangement of protoxylem<sup>1</sup>; it has the phloem slightly interrupted.

*T. reptans*, Swartz, and *T. Filicula*, Bory, have a very small xylem-group, of five or six tracheides surrounded by phloem.

*T. pallidum*, Blume, has a small xylem-band with the protoxylem possibly immersed, as seen in *Hymenophyllum dilatatum* in the specimen shown in Fig. 21. Two species of *Trichomanes* among the series examined were found to have

<sup>1</sup> Prantl, l. c.



collateral structure in the rhizome, viz. *T. muscoides*, Swartz, and *T. membranaceum*, Linn.<sup>1</sup> The xylem consists of a small group of tracheides (6–8), of which the small ones representing protoxylem lie towards the lower side; the phloem occurs on the upper side of the stele only. Fig. 41 is a transverse section of the rhizome of *T. muscoides*. This structure, though very much more delicate, resembles the monarch stele of the root of *Ophioglossum vulgatum*; a corresponding structure thus appears to have been produced by reduction from two entirely different structures, namely from a xylem-ring with central protoxylem, and surrounded by phloem in the one case, and a typical diarch root-structure in the other case. A case of greatly reduced structure is described by Giesenhagen<sup>2</sup> in the rhizome of a species of *Trichomanes* described as *T. microphyllum*, Ghgn., but in a later paper<sup>3</sup> united with *T. labiatum*, Jenman. In this species the endodermis surrounds a bundle consisting of nothing but one tracheide surrounded by four to five parenchymatous cells. A still further reduction is found in *T. Motleyi*, described by Karsten<sup>4</sup>; in this plant the stem possesses no tracheides, but only a few conjunctive parenchyma-cells. The sterile leaf has no tracheides, but the fertile leaf has a true vascular bundle.

In *T. muscoides* the petiolar bundle is practically identical in structure with the stele of the rhizome, both being collateral and of about the same size. In the species with sub-collateral structure in the rhizome, the petiolar structure differs only in the interruption of the phloem; being collateral.

The above description of different species of the genus *Trichomanes* shows a considerable range of structure in the stele; the chief types being:—

1. A ring of xylem surrounding a fair-sized mass of parenchyma, which contains the protoxylem. *T. reniforme*.

<sup>1</sup> Prantl describes collateral structure of the rhizome in his section *Hemiphlebiium* of the genus *Trichomanes* (l. c., p. 26).

<sup>2</sup> Giesenhagen, *Flora*, 1890, p. 445.

<sup>3</sup> Giesenhagen, *Flora*, 1892, *Ergänzungsband*, p. 178.

<sup>4</sup> Karsten, *Epiphytenformen d. Molukken*, *Ann. Jard. Bot. Buitenzorg*, XII, p. 135.

2. A solid mass of xylem with internal protoxylems accompanied by only scanty parenchyma. *T. radicans*, *T. Prieurii*.

3. The sub-collateral type. *T. trichoideum*.

4. The collateral type. *T. muscoides*.

5. Only one tracheide or no tracheide, and no phloem present. *T. labiatum* and *T. Motleyi*.

6. A solid mass of xylem with scattered indefinite protoxylem. *T. spicatum*.

7. A solid mass of xylem with distinct peripheral protoxylem. *T. scandens*.

Of these types, 4, 5, 6 and 7 do not occur in *Hymenophyllum*. A comparison with the latter genus, where transitional forms between types 1 and 3 occur, suggests that in *Trichomanes* also the sub-collateral structure has probably been derived from the two-banded type (*T. reniforme*) by reduction, which has here gone still further and produced collateral structure in *T. muscoides*. As to the remaining types, it may appear doubtful whether they are to be regarded as more primitive than *T. reniforme* or more specialized forms.

In the petiolar bundle simple collateral structure is found in small forms of both genera, in larger forms in both genera also, the xylem is an arc with a protoxylem at the two ends, and the xylem arc closes to a ring in the base of the petiole. Larger bundles with three protoxylems are found in *Trichomanes* only.

#### PHYLOGENY, &c.

With regard to the phylogenetic questions in *Hymenophyllum* and *Trichomanes*, several views may be suggested, e.g.

1. That the solid stele (as in *T. scandens*) is primitive, that the type of *T. reniforme* was derived from this (by the replacing of many of the central tracheides by parenchyma), and that further specialization took place in the direction of reduction, ending with collateral structure; that the genus *Hymenophyllum* is a series of reduction parallel with what is found in *Trichomanes*, and that, as its most complicated

type resembles *T. reniforme*, the more bulky forms with a solid stele have died out.

Or 2. That in *Trichomanes* the structure of *T. reniforme* is primitive and that specialization went on in two directions, namely reduction of conducting elements, leading to collateral structure in *T. muscoides*, and increase in conducting elements, leading to types with a large stele, e. g. *T. radicans*, *T. scandens*, &c.; while in *Hymenophyllum*, starting with a structure like that of *T. reniforme*, as found in the larger forms, specialization took place only in the direction of reduction to the sub-collateral type.

Or 3. That the collateral type is primitive (as held by Prantl), and specialization involved complication and increase in the size of the stele in both genera<sup>1</sup>.

Of these, the second seems in some ways the most probable.

The type of structure found in *T. reniforme* and in the similar species of *Hymenophyllum* is evidently specialized. The somewhat dorsiventral differentiation of the xylem, with a lower band developed early for the attachment of roots, is an adaptation to the creeping habit. The forms with upright stems do not appear to form a series by themselves, but species nearly allied to one another may differ in that the stem is upright in one and creeping in another, and this may occur in different sections of the genus<sup>2</sup>. Among the dimorphic forms of *Trichomanes*, *T. spicatum* and *T. heterophyllum* differ in this way.

Though the Hymenophyllaceae may have been derived originally from ancestors with upright stems, it is quite possible that all the living members of the order may have been evolved from forms with a creeping rhizome, some species retaining this character, and others having changed to upright habit, others possibly having progressed from creeping to upright habit and back again<sup>3</sup>. When the upright habit leads, as it usually does in this order, to short internodes and a slower-growing stem, the protoxylem will be liable to

<sup>1</sup> Prantl, l. c., p. 59, &c.

<sup>2</sup> Prantl, l. c., p. 58.

<sup>3</sup> This is also Prantl's view.



become less definite ; and being formed later with regard to the region of elongation, it may cease to consist of spiral elements. The mass of parenchyma accompanying it may be reduced (as it probably has some function connected with the typical protoxylem), so that the stele becomes a nearly uniform mass of tracheides and parenchyma. This is seen in *T. spicatum*, and as this species has its fertile fronds different from its vegetative fronds, some probability is given to the view that its structure also is not primitive. *T. heterophyllum* also has dimorphic leaves and is classed next to *T. spicatum* in Hooker and Baker's Synopsis, but it differs from the former species in having a creeping rhizome. In structure, its rhizome appears to be almost the same as the stem of *T. spicatum*. These facts probably mean that *T. heterophyllum* is a species that has returned from upright to creeping habit, its structure having remained as in *T. spicatum*.

*T. Prieurii* is an upright form with a solid stele in its stem containing nearly uniformly arranged tracheides and parenchyma. Its protoxylems, however, are spiral and quite definite for a short distance after entering from the leaf, but as far as could be determined in rather scanty material, they gradually die out in passing downwards. Their spiral character is probably due to greater elongation of the stem after their formation than in *T. spicatum*.

To return to the type of *T. spicatum*, where the first found elements of the stele are scattered, some perhaps represent spiral protoxylem of an earlier type of plant, others probably do not, and under new conditions of growth a new protoxylem of spiral elements might arise in a different region of the stele. This is the theory put forward to explain the structure of the stele in *T. scandens*. The stele here is of the solid kind found in upright species like *T. spicatum*, but there is a definite peripheral spiral protoxylem. This species may be regarded as having perhaps given up an upright habit for its present creeping one, and developed a new peripheral protoxylem of spiral elements on account of its more rapid growth and greater elongation of its internodes.

All the Hymenophyllaceae being filmy, there are no stomata in the lamina, and further, as far as is known, stomata do not occur in any part of the plant<sup>1</sup>. It is most convenient to apply the word 'filmy' to leaves which possess no intercellular spaces, rather than to reserve it for leaves whose lamina is strictly one cell thick. In the above sense *T. reniforme* is filmy, though the lamina is four cells thick. The absence of intercellular spaces gives to a leaf a translucent appearance, and from the external appearance it is hardly possible to distinguish a leaf that is one cell thick from one that is two cells thick.

As the Hymenophyllaceae have probably been derived from Ferns that were not filmy, it was possible that abortive stomata might be found, but a search for them gave negative results. The cells (*a* and *b*) shown in Fig. 43 bear a certain resemblance to the guard-cells of a rudimentary stoma, but this is probably accidental. It was the only case of the kind met with, and occurred in an early leaf of a plant of *T. Bancroftii*. Some cases of filmy Ferns outside the Hymenophyllaceae, namely *Todea superba*, Col., *T. hymenophylloides*, Rich. and Less., &c., and *Asplenium resectum*, Sm.<sup>2</sup>, were examined for comparison. All their leaves appeared to be destitute of stomata. Subsequently, however, some very young 'seedlings' of *T. hymenophylloides*, with the first two leaves only, were found connected with their prothalli. Prothalli, mostly without young sporophytes, were resting on the upper side of the leaves of a plant of this species, which had abundant mature sori on the lower side of its leaves. The conditions were such as to make it practically certain that the prothalli belonged to the plant on which they were found. Some prothalli had been formed by the germination of spores in their sporangia, which were ruptured but still arranged in sori on the lower side of the leaf. This occurred where part of a leaf had become turned upside down. Then again, where one leaf rather closely overhung another, sporangia and spores had fallen on to the surface of the lower leaf, and it was leaves

<sup>1</sup> Prantl, l. c., p. 23.

<sup>2</sup> See Bower, *Annals of Botany*, III, p. 348.

thus placed that also had prothalli resting on them, the prothalli agreeing well in appearance with those produced by germination of spores *in situ*. The evidence cannot be called conclusive that the prothalli and young sporophytes belonged to *T. hymenophylloides*, but it is very nearly so. The reason for which these are mentioned is that the first two leaves were not filmy, but had numerous intercellular spaces and stomata. Unfortunately none of the sporophytes had advanced further to form filmy leaves. Some 'seedlings' of *Todea Fraseri*, Hook. and Grev., and *T. hymenophylloides* supplied by Messrs. Backhouse, did not agree with the above, their first leaves being entirely filmy. This would make one doubt the identity of the other seedlings, but it is quite possible that when the prothalli and young sporophytes are grown under normal conditions, the first leaves are filmy, but that when grown under the abnormal conditions of the first case, the first leaves might show signs of reversion.

Some of the leaves of *Asplenium resectum*, the other filmy plant examined, possessed evident abortive stomata. The mother-cell of the stoma had in some cases remained undivided (as in Fig. 45, *m.c.*) and in other cases it had undergone division into two cells, resembling guard-cells, but of course destitute of a pore (Fig. 44, *g*). The lamina in this species is mostly two cells thick. This species is further interesting because some specimens are filmy, while others have leaves with abundant stomata and intercellular spaces. The filmy form is separated as var. *udum*, Clark, so that the filmy character is here not even of specific value.

For *Asplenium resectum* it is clear that the filmy habit is a modification of the normal type of leaf, as evidenced by the functionless rudiments of stomata. In *Todea*, accepting the seedlings mentioned as belonging to *T. hymenophylloides*, the same appears to be the case; but in the Hymenophyllaceae similar evidence is at present wanting as to whether the filmy habit is primitive or reduced. As, however, the anatomy of the stem appears to point to a series of reduction, it is probable that the filmy habit is secondary and not primitive.



Another case of normal leaves (with stomata) and filmy leaves occurring in different specimens of the same species is described by Giesenhagen<sup>1</sup> in *Asplenium obtusifolium*, Linn. He comes to the conclusion that in one variety ( $\alpha$ ) the filmy habit is fixed, but that in another ( $\beta$ ), stomata and intercellular spaces occur in the larger specimens, but are sometimes quite absent in the smallest. In this species, as in *A. resectum*, the filmy forms are those which grow in the dampest localities. Giesenhagen<sup>2</sup> regards the simple (filmy) structure of the Hymenophyllaceae as having become hereditarily fixed, as in the variety of *Asplenium obtusifolium* mentioned, the plants being adapted to an extremely damp habitat. They have become, as he says, practically water-plants, and can absorb water by their leaves, so that the conducting elements are naturally reduced.

In criticizing Prantl's view that the species of *Trichomanes* with the simplest structure (Prantl's *Hemiphlebicae*) are the most primitive, Giesenhagen<sup>3</sup> rightly points out that the pseudo-veins which are found in these species are clearly reduced veins; and that therefore the species are reduced; and further that the rarity of roots in several of these species must also be regarded as a case of reduction; because in the only species where the embryo has been investigated (although they unfortunately do not belong to the *Hemiphlebicae*)<sup>4</sup> a primary root is formed.

It will be as well to recapitulate some of the foregoing. *Asplenium resectum*, &c., show how the filmy structure may be derived from normal Fern-structure. \* The filmy character, leading to semi-aquatic life, will cause reduction in structure. Hymenophyllaceae are filmy, and show signs of reduction (e.g. pseudo-veins and rarity of roots in some of their simpler forms), therefore they are probably reduced from normally constructed Ferns, the simplest forms being the most reduced. As in *Hymenophyllum* and *Trichomanes*, series of reduction

<sup>1</sup> Giesenhagen, Ueber hygrophile Farne, Flora, Ergänzungsbd., 1892, p. 159.

<sup>2</sup> l. c., p. 174.

<sup>3</sup> Giesenhagen, Die Hymenophyllaceen, Flora, 1890, p. 437.

<sup>4</sup> Giesenhagen, Flora, 1890, Taf. XVI, Fig. 17, *Trichomanes alatum*.

occur which are similar to one another, they appear to be parallel genera. As the smaller forms (sub-collateral types) are the most reduced in each, the larger (two-banded) forms are the more primitive. The species of *Trichomanes* with a solid circular stele (of a type hardly represented in *Hymenophyllum*) might be regarded as more primitive in the series of reduction than *T. reniforme* and the larger *Hymenophyllums*, but many of them have a subcoriaceous frond, so they have probably become adapted to life in slightly less damp localities than the other species<sup>1</sup>, which would necessitate the differentiation of a stouter stele in equal-sized plants. *T. radicans*, though it is not subcoriaceous, spreads to temperate regions, where the saturation of the air is not likely to be of the same degree as in the tropics. Hence the conduction from the soil would need to be greater, as evidenced by the size of its stele. Its structure points to its having been derived from a two-banded type. This species is much nearer to *T. reniforme* in structure than are the other species with a solid stele (e.g. *T. scandens*, *T. spicatum*); and as *T. reniforme* appears more primitive than *T. radicans*, on account of sori occurring on most of its veins<sup>2</sup>, it seems probable that all the forms with a solid stele owe their type of structure to specialization. It has been pointed out that a change to a structure like that of *T. spicatum* might be favoured by a change to the upright habit with a short stem, and this would account for its being found in *T. spicatum* and *T. Bancroftii*. In a return to the creeping habit, this structure might be retained as in *T. heterophyllum*, or modified as in *T. scandens*.

Among fossil plants the Botryopterideae have been pointed out as showing structural resemblance to the Hymenophyllaceae<sup>3</sup>. To quote from the work just mentioned in the note :

<sup>1</sup> Giesenhagen, Flora, 1890, p. 419, 'The few Hymenophyllaceae, which live outside the primeval forest, are less sensitive to want of water.' Unfortunately there are not sufficient data of the exact habitats of different species.

<sup>2</sup> Prantl, l. c., p. 7.

<sup>3</sup> Scott, Studies in Fossil Botany, p. 298. (The author kindly allowed me to refer to the proof-sheets of this work.) 'The resemblance between the anatomy of one of the larger species of *Trichomanes* and that of *Zygopteris* is in fact very striking.'

'The leaf-trace [in *Trichomanes*] gives off the stele of the axillary branch precisely in the same manner as in *Zygopteris*, the resemblance extending even to details.' This is seen by comparing figures of *Zygopteris scandens*, Stenz.<sup>1</sup>, with the diagrams of the node of *T. radicans*. The following facts are taken from Scott's work quoted above<sup>2</sup>. In *Zygopteris* the wood in section has the outline of an irregular five-rayed star, the five rays corresponding with the orthostichies of a two-fifths phyllotaxis. In the wood there is a central five-rayed tissue (of parenchyma including small tracheides) covered by a peripheral zone composed mostly of large scalariform tracheides but with smaller tracheides at the ends of the arms. The leaf-trace passes off as a closed ring of tracheides enclosing central parenchyma and small tracheides. In comparing the above structure with the Hymenophyllaceae, if the small central tracheides are protoxylem, the agreement with *Trichomanes reniforme* or the larger *Hymenophyllums* is very close, the difference being such as would be due to a two-fifth arrangement on the one hand and distichous on the other. The annular xylem of the base of the leaf-trace also agrees with *T. reniforme*. *Botryopteris* has a solid xylem-mass (consisting of tracheides only) in its stem. The Botryopterideae mentioned are found in the Coal-Measures and Permian. The sporangia of neither genus agree with the Hymenophyllaceae as to their annulus, therefore close affinity cannot be affirmed; but quite possibly Botryopteridean structure may have been shared by the members of a rather large generalized group, from some of which the Hymenophyllaceae have been derived.

In *Botryopteris forensis* 'the tissue of the leaf appears to have been fleshy; on the one surface numerous stomata were present<sup>3</sup>.' Thus fossil plants occur which resemble the Hymenophyllaceae (more than any living Ferns do) in several structural points, but were not filmy; i.e. the only fossil evidence (not very weighty, it is true), does not support the

<sup>1</sup> Stenzel, Die Gattung Tubicaulis, Bibliotheca Botanica, Cassel, 1889, Taf. VII, Figs. 60, 61, 65.

<sup>2</sup> Scott, l. c., p. 280 et seq.

<sup>3</sup> Renault, cited by Scott, l. c., p. 294.



view of the filmy habit being primitive. The solid stele of *Botryopteris*, as the wood consists of tracheides only, resembles the stele found in the lower part of the seedling-stem of *Trichomanes* rather than the solid stele of *T. spicatum*, &c.

The transition from root to stem structure has been investigated by Leclerc du Sablon<sup>1</sup> in a young plant of *Trichomanes alatum*. Described shortly, these are his results:—The root is diarch with no pith; at a distance from the prothallus a pith may appear in the primary root, or may not; in the passage from root to stem, the number of tracheides increases, and the wood then forms a rounded mass completely surrounded by phloem<sup>2</sup>; in the larger parts the tracheides increase further in number, then separate a little from one another, so that the whole of the wood is formed of tracheides mixed with unligified parenchymatous cells; apart from further changes in thickness, the stele has then attained its mature structure. The stem of the young plant thus has a solid xylem-mass similar to *T. spicatum* or *T. scandens*.

These developmental facts may mean that the solid xylem-mass is primitive, but it does not prove that the species may not phylogenetically have passed through a stage in which the mature stem may have been similar to that of *T. reniforme* or *H. scabrum*. *T. alatum* and other species with a solid stele may be cases in which the youth-form of structure is retained in the mature plant.

In view of the probability (owing to varying conditions) that reduction has taken place in different series, especially in the genus *Trichomanes*, and that in this genus the change from creeping to upright habit, and perhaps to coriaceous habit has occurred, nothing weighty can be put forward with regard to the classification of the species. But a few facts may be pointed out.

The two-banded *Hymenophyllums* agree well with one

<sup>1</sup> Leclerc du Sablon, Recherches sur la formation de la tige des Fougères, Ann. des Sci. Nat., 7<sup>e</sup> sér., t. xi, p. 11

<sup>2</sup> l. c., Fig. 21. There is here no pith, and no parenchyma among the tracheides.

another in details, and this supports the classification of *H. demissum*, *H. scabrum*, and *H. dilatatum* close together in the same section of the genus in Hooker and Baker's Synopsis. *H. flabellatum* is there placed next to *H. scabrum*, which fits in with the structure of the former being apparently derived from the two-banded type. In *Trichomanes*, *T. scandens*, *T. rigidum*, *T. apiifolium*, *T. Prieurii*, and *T. ericoides* are classed together (with other species not examined) in the last section of the genus. This agrees well with their structure. *T. spicatum* with *T. heterophyllum* are classed in a different section from *T. scandens*, &c., and *T. Bancroftii* with *T. alatum* are placed in a third section. These four species might structurally form part of the same series as the above (*T. scandens*, &c.). Hence the upright habit may have been assumed in three different series.

Prantl<sup>1</sup> expresses his opinion of the development of the Hymenophyllaceae in the form of a phylogenetic tree, and represents *Hymenophyllum* and *Cardiomanes* (i. e. *Trichomanes reniforme*) as springing independently from the base of the series, the other subdivisions of *Trichomanes* being lateral branches of other series. Prantl was unable to examine the anatomy of *T. reniforme*, and in *Hymenophyllum* appears to have examined only the simple types<sup>2</sup>. This partly accounts for his regarding the simpler structures (sub-collateral, &c.) as the more primitive.

Bower's interesting results in spore-counting<sup>3</sup> show that *Trichomanes reniforme* agrees with *Hymenophyllum* in forming a large number of spores per sporangium, while other species of *Trichomanes* have a low number. It is very interesting, therefore, that *Trichomanes reniforme* resembles some of the *Hymenophyllums* rather closely in structure, but differs in this from other species of *Trichomanes*. Bower regards the low number of spores in *Trichomanes* as being attained by

<sup>1</sup> Prantl, l. c., p. 58.

<sup>2</sup> With the exception of *H. flabellatum*, in which he remarks that the protoxylem strangely lies in the middle of the strand.

<sup>3</sup> Bower, The Leptosporangiate Ferns, Phil. Trans., 1899, p. 64.

reduction<sup>1</sup>, and the Hymenophyllaceae as forming a sequence, of which species of *Hymenophyllum* are less extreme types than species of *Trichomanes*<sup>2</sup>. Thus species of *Hymenophyllum* with *T. reniforme* are comparatively primitive, while other species of *Trichomanes* are more specialized. This is hinted at in the anatomy, because, though sub-collateral structure occurs in both genera, further reduction to the collateral type appears to occur in *Trichomanes* only, and in the latter genus signs of transition between the two-banded and sub-collateral types have disappeared.

The relationships of the Hymenophyllaceae will be referred to in the later paper.

As the literature has been insufficiently referred to above, some of the more important works, which deal with the anatomy of the Hymenophyllaceae, will now be quoted.

Von Mohl<sup>3</sup> mentions that in *Hymenophyllum* and *Trichomanes* the vessels are all united into a central bundle. Presl<sup>4</sup> gives a series of diagrams illustrating the arrangement of the vascular bundles in the petiole of a large number of Ferns, and represents all the Hymenophyllaceae given as having a circular vascular bundle. Mettenius<sup>5</sup> refers to the occurrence and position of axillary buds in a large number of species. He describes the pseudo-veins (*Scheinnerven*), and the presence of prosenchyma in the petiolar bundle of one or two species of *Trichomanes*. Of the central strand in the stem of the Hymenophyllaceae, he says that it is composed chiefly of scalariform elements, and contains also annular and spiral elements, always at the periphery, and often also in the middle, and, besides these and the scalariform elements, elongated thin-walled cells.

<sup>1</sup> l. c., p. 65.

<sup>2</sup> l. c., p. 66.

<sup>3</sup> Von Mohl, Ueber den Bau des Stammes der Baumfarne, Vermischte Schriften, 1845, p. 115.

<sup>4</sup> Presl, Die Gefässb. im Stipes der Farne, Abh. k. Böhm. Ges., v. 5, 1847, Tab. 5.

<sup>5</sup> Mettenius, Hymenophyllaceen, Abh. k. Sächs. Ges., vii, 1864, and Ueber Seitenknospen bei d. Farnen.



Russow<sup>1</sup> gives a rather general description of the anatomy of stem and petiole in the Hymenophyllaceae. In the stem he mentions that the tracheides form a compact mass in some species, but are separated from one another by conjunctive parenchyma (*Geleitzellen*) in others. The protoxylem-elements are given as being scattered in numerous groups over the whole transverse section of the xylem, and it is represented so in a diagram of *Trichomanes radicans* (Taf. X, Fig. 6). The outer phloem-elements are regarded as being probably protophloem, and the roots are described as varying from monarch up to octarch. The elements like bast-fibres, which occur in petiolar bundles of *T. Prieurii*, &c., are regarded as morphologically similar to those in some Gleicheniaceae and Schizaeaceae. Prantl<sup>2</sup> devotes much attention to the venation of the leaves in the Hymenophyllaceae, and comes to the conclusion that a study of their habit points to the development having proceeded from simple plants with small undivided leaves to the more complex types. Several of Prantl's anatomical results have been referred to above. Potonié<sup>3</sup> states that in *Hymenophyllum nitens* the common origin of endodermis and pericycle (several cells thick) is placed beyond doubt by the arrangement of the cell-walls in the mature rhizome. He regards the protophloem of Hymenophyllaceae as being very probably sieve-tubes. In *H. nitens* he finds 'Lückenparenchym' present. The structure of the petiole is described in a large number of Ferns by Thomae<sup>4</sup>. In describing *T. radicans*, he mentions that in the lower part of the petiole there is a round vascular bundle, with annular xylem, but that, further up, the ring opens on the upper side, and the bundle becomes heart-shaped. He states that the part shut in by the ring consists only of parenchymatous cells, so like Prantl he overlooked the internal tracheides.

<sup>1</sup> Russow, Vergleich. Untersuch. d. Leitbündel-Kryptogamen, Mém. de l'Acad. Imp. de St. Pétersbourg, vii, 19, 1872, p. 94.

<sup>2</sup> Prantl, Unters. z. Morph. d. Gefässkryp., I. Hymenophyllaceen, 1875.

<sup>3</sup> Potonié, Zusammensetzung der Leitbündel, Jahrb. d. k. bot. Gart. Berlin, II, 1883.

<sup>4</sup> Die Blattstiele der Farne, Pringsheim's Jahrb., xvii, 1886.

Thomae describes the petiolar bundle of *H. tunbridgense* correctly. Two of Giesenhagen's papers have been referred to above. In the earlier paper<sup>1</sup> he puts forward the view that the function of the vascular bundles in the smaller members of the Hymenophyllaceae is not for the supply of water to the assimilating tissue, but partly, at any rate, for the receptacle. In Van Tieghem's *Traité*<sup>2</sup>, the stems of *Trichomanes* and *Hymenophyllum* are given as examples of the occurrence together of centripetal and centrifugal wood, the centripetal wood beginning towards the middle of the radius, and possessing centrifugal wood outside it. This description would not apply to any sub-collateral types nor to *T. scandens*. It would serve for species with more than one protoxylem-group embedded in the metaxylem, but, if one regards the region of the stem of *H. scabrum*, where there is only one protoxylem, it would not hold good. There is in this case no centripetal wood; the first-formed among the protoxylem-elements are central to the others. Poirault<sup>3</sup> makes a comparison of the structure in the young stem of *Trichomanes* with that of the node of *Gleichenia*. He refers to the researches of Leclerc du Sablon on the young plant of *T. alatum*, and it is not quite evident from his description whether his data are taken entirely from Leclerc du Sablon's work, or whether he has made a further investigation of *Trichomanes* himself. If the former, he appears to have confused Leclerc du Sablon's description of *T. alatum* with that of an entirely different type. Leclerc du Sablon makes no mention of the appearance of sieve-tubes within a pith in *T. alatum*, and it is on this point that the comparison is founded. The presence of internal sieve-tubes certainly seems very improbable.

<sup>1</sup> Giesenhagen, Die Hymenophyllaceen, Flora, 1890, p. 447.

<sup>2</sup> Van Tieghem, Traité de Botanique, second edition, 1891, p. 763.

<sup>3</sup> Poirault, Recherches sur les Cryp. Vasc., Ann. des Sci. Nat., 7<sup>e</sup> sér., t. xviii, 1893, p. 177.

# SUMMARY.

Some of the structural characters of the Hymenophyllaceae will now be summarized.

The stem is monostelic, and one leaf-trace passes off to each leaf. The stele contains no pith, and is of several types, e.g. :—

1. A xylem-mass with internal protoxylems, connected with leaf-traces.
2. A xylem-mass with indefinite scattered protoxylem.
3. A xylem-mass with peripheral protoxylem.
4. Sub-collateral.
5. A collateral bundle.

The axillary branch is attached to the leaf-trace.

The petiolar bundle is usually collateral, and very similar to the stele in the case of the sub-collateral and collateral types.

In other types it often resembles stem-structure in its lower region, and higher up it usually has an arched xylem with two protoxylems at the ends, or with an additional one at the median dorsal point.

As shown by *Hymenophyllum*, sub-collateral structure has probably been derived from a more complicated type by reduction. The filmy habit is probably not primitive.

*T. reniforme* agrees more closely in structure with *H. scabrum*, &c., than with any species of its own genus.

The investigations, which form the subject of the present paper, have been carried out at the suggestion of Dr. D. H. Scott, F.R.S., to whom I wish to express my thanks for his valuable advice throughout the work. I am also indebted to Mr. J. G. Baker, F.R.S., and Mr. C. B. Clarke, F.R.S., for their kind assistance in some systematic points, and to Mr. C. H. Wright, A.L.S., in this and in other ways. The material used in a considerable number of species was from living plants grown in the Royal Gardens, Kew.



## EXPLANATION OF FIGURES IN PLATES XXV, XXVI, AND XXVII.

Illustrating Mr. Boodle's paper on the Anatomy of the Hymenophyllaceae.

The following lettering is used in most of the illustrations:—*sc.*, sclerenchyma; *e.*, endodermis; *ph.*, phloem; *pph.*, protophloem; *px.*, protoxylem; *x.*, metaxylem; *u.*, upper xylem-band; *l.*, lower xylem-band; *r.*, tracheides connected with a root. Where *x*<sup>1</sup>, *px*<sup>1</sup>, *ph*<sup>1</sup> are used they refer to the metaxylem, protoxylem, and phloem respectively of a petiole or leaf-trace.

Figs. 22 and 24 are reproduced from photographs by Dr. E. C. Bousfield of sections prepared by Mr. D. T. Gwynne-Vaughan.

### PLATE XXV.

Fig. 1. Diagram of transverse section of the rhizome of *Hymenophyllum scabrum*. The lump at the left-hand end of the lower xylem-band is connected with a root.

× 55.

Fig. 2. Transverse section of stele of young rhizome of *H. scabrum*. × 190.

Fig. 3. Transverse section of stele of older rhizome of *H. scabrum*. × 190.

Fig. 4. Transverse section of stele of mature rhizome of *H. scabrum*. × 190.

Fig. 5. Diagram of transverse section of rhizome of *H. demissum*, var. *nitens*.

× 45.

Fig. 6. Diagram of transverse section of petiole of *H. dilatatum*, var. *Forsterianum*. × 50.

Fig. 7. Transverse section of vascular bundle of petiole of *H. dilatatum*, var. *Forsterianum*. × 260.

Fig. 8. Transverse section of vascular bundle of petiole of *H. dilatatum*, var. *Forsterianum*, a short distance above axillary branch. × 385.

Fig. 9. Transverse section of node of *H. dilatatum*, var. *Forsterianum*. *s.*, stele of rhizome; *lt.*, leaf-trace; *br.*, axillary branch. × 90.

Fig. 10. Transverse section of node of *H. dilatatum*, var. *Forsterianum*. Stele of rhizome on the right, leaf-trace on the left. × 200.

Fig. 11. Transverse section of node of *H. dilatatum*, var. *Forsterianum*. Stele on the left, and leaf-trace on the right partly fused. × 200.

### PLATE XXVI.

Fig. 12. Transverse section of node of *H. dilatatum*, var. *Forsterianum*; further stage of fusion. Stele of rhizome on right, leaf-trace on left. × 200.

Fig. 13. Transverse section of rhizome of *H. sericeum*. × 260.

Fig. 14. Transverse section of rhizome of *H. fucoides*. × 235.

Fig. 15. Transverse section of petiole of *H. fucoides*. × 235.

Fig. 16. Transverse section of rhizome of *H. tunbridgensis*. × 380.

Fig. 17. Transverse section of petiole of *H. tunbridgensis*. × 390.

Fig. 18. Transverse section of rhizome of rather large plant of *H. cruentum*. × 390.

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Fig. 19. Transverse section of rhizome of smaller plant of *H. cruentum*. Lower xylem-band probably absent.  $\times 390$ .

Fig. 20. Transverse section of petiole of *H. cruentum*. *s.t.* is a sieve-tube in contact with a tracheide.  $\times 390$ .

Fig. 21. Transverse section of stele of rhizome of small plant of *H. dilatatum*.  $\times 165$ .

Fig. 22. Photograph of transverse section of rhizome of *Trichomanes reniforme*.  $\times 75$ .

Fig. 23. Transverse section of young rhizome of *T. reniforme*.  $\times 260$ .

Fig. 24. Photograph of transverse section of rhizome of *T. radicans*.  $\times 75$ .

Fig. 25. Transverse section of young petiole of *T. radicans*.  $\times 200$ .

PLATE XXVII.

Fig. 26. Diagram of transverse section of petiolar bundle of *T. radicans*. As in previous diagrams, the phloem is indicated by broken lines, the protoxylem by dots, and the metaxylem by cross-hatching.  $\times 95$ .

Fig. 27. Diagram of transverse section of base of petiolar bundle of *T. radicans*. The xylem here forms a continuous ring.  $\times 95$ .

Fig. 28. Longitudinal section of node of *T. radicans*. *br.*, stele of axillary branch; *pet.*, bundle of petiole.  $\times$  about 6.

Fig. 29. Diagram of transverse section of node of *T. radicans*. *lt.*, leaf-trace; *br.*, axillary branch; *s.*, stele of rhizome; protoxylem, &c. indicated as before.  $\times$  about 40.

Fig. 30. Diagram of same node lower down. Leaf-trace and stele of axillary branch fused.  $\times$  about 40.

Fig. 31. Diagram of same node. Fusion of leaf-trace and stele of rhizome beginning.  $\times$  about 40.

Fig. 32. Diagram of same node. Fusion complete, except that the two protoxylems remain distinct.  $\times$  about 40.

Fig. 33. Diagram of transverse section of node of *T. Prieurii*. In this and the three succeeding diagrams the sclerenchyma-groups are shaded; *x*, *x'*, *x''* are the xylems of stele of rhizome, of first leaf-trace, and second leaf-trace respectively. *a* is an axillary branch, and *r* a root-bundle cut obliquely.  $\times 12$ .

Figs. 34, 35, and 36. Diagrams of the same node at successively lower levels, each.  $\times 12$ .

Fig. 37. Enlarged diagram of the stele of the stem shown in Fig. 33. The sclerenchyma is shaded, and the protoxylem represented by dots.  $\times 90$ .

Fig. 38. Transverse section of the stele of *T. scandens*.  $\times 140$ .

Fig. 39. Transverse section of young petiole of *T. scandens*, showing three protoxylems.  $\times 260$ .

Fig. 40. Photograph of transverse section of petiolar bundle of *T. apiifolium*.  $\times$  about 50.

Fig. 41. Transverse section of rhizome of *T. muscoides*.  $\times 480$ .

Fig. 42. Bordered pits of tracheide of *T. Bancroftii*, exceptional in form.  $\times 1120$ .

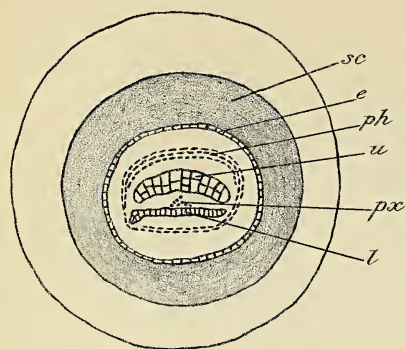
Fig. 43. Stoma-like structure in early leaf of *T. Bancroftii*.  $\times 90$ .

Fig. 44. Abortive stoma on filmy leaf of *Asplenium resectum*; *g.*, guard-cells.  $\times 255$ .

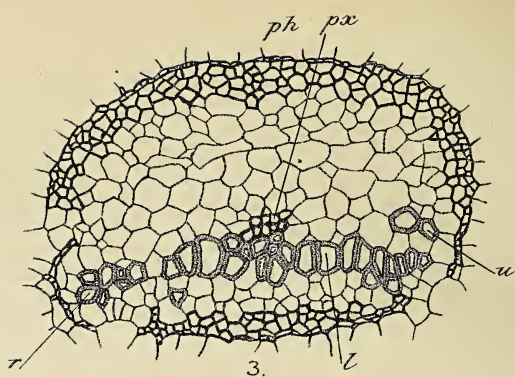
Fig. 45. Abortive stoma on the same leaf; *m.c.*, mother-cell of stoma.  $\times 255$ .



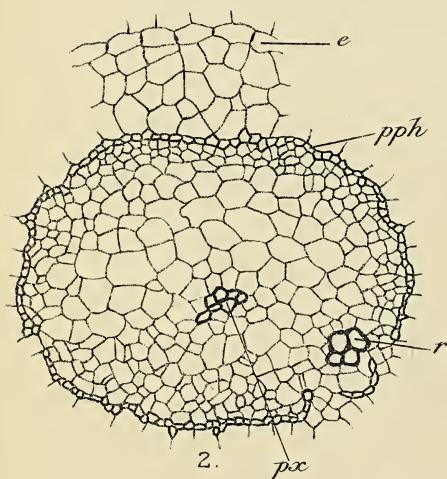




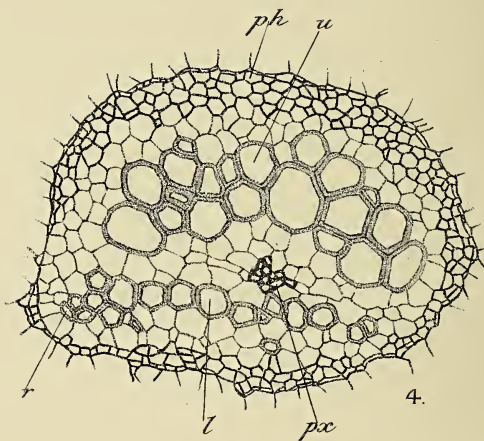
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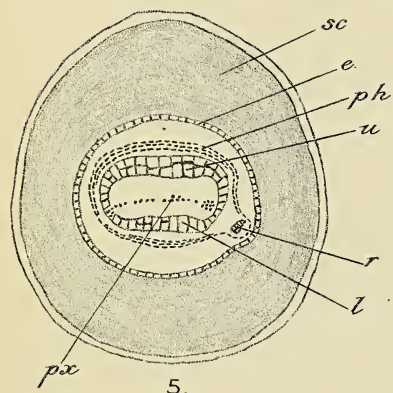
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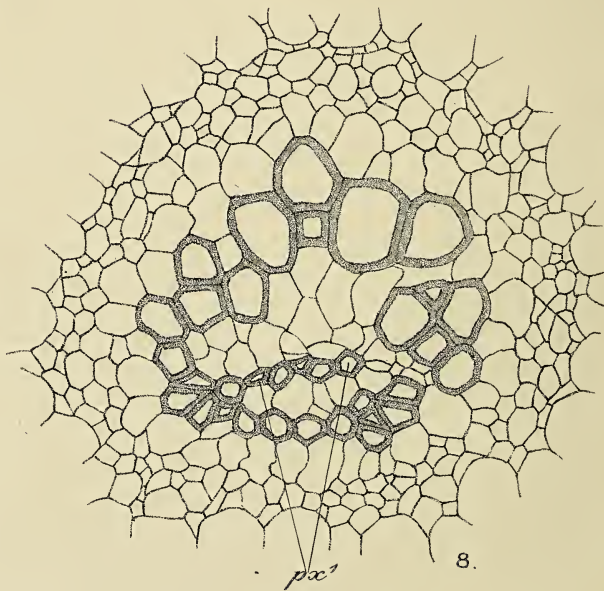
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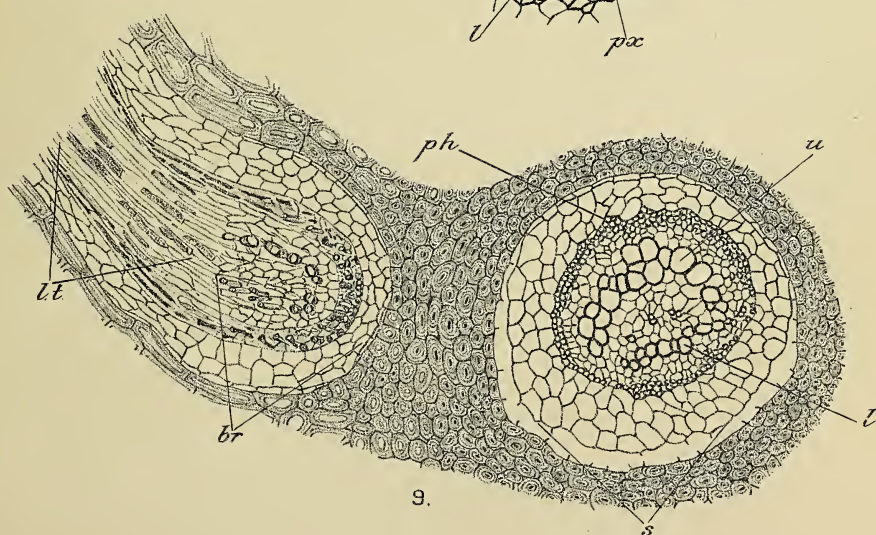
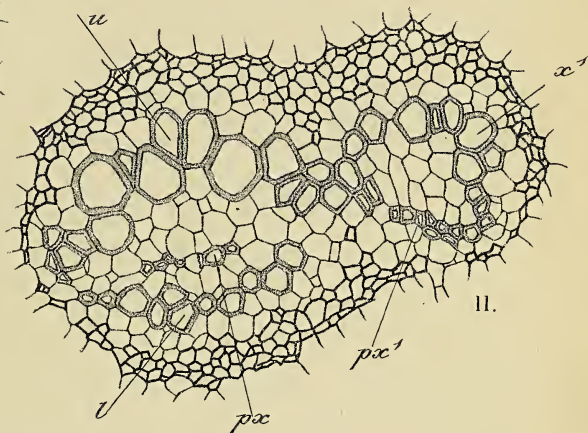
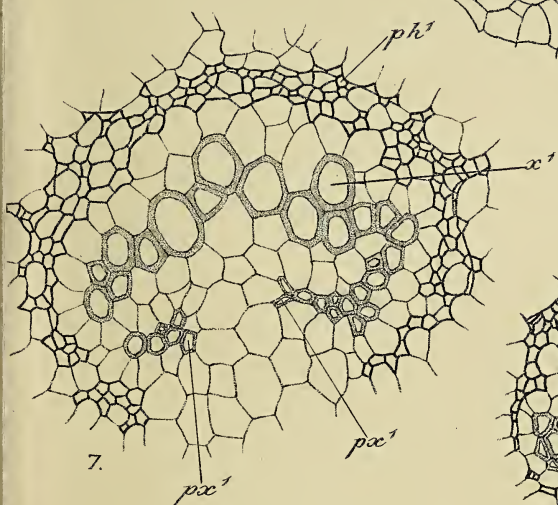
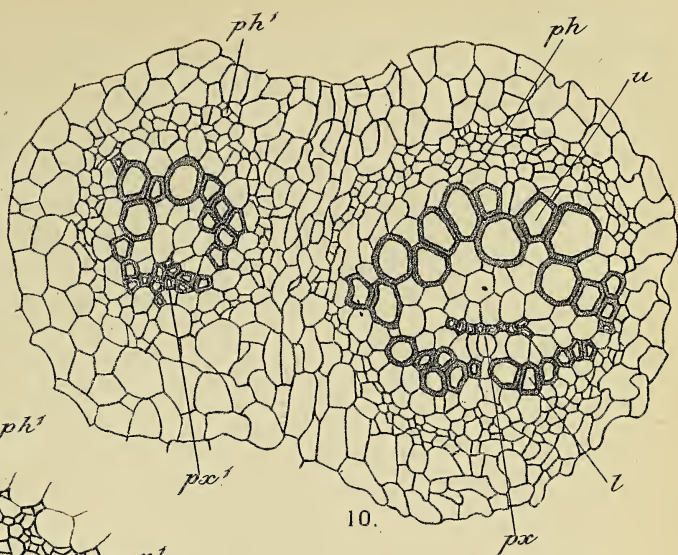
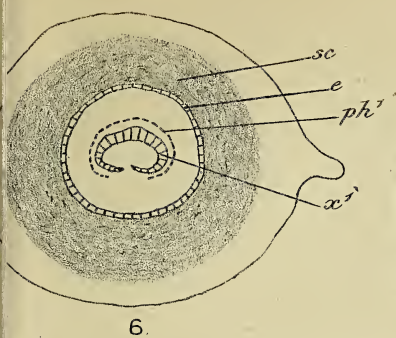
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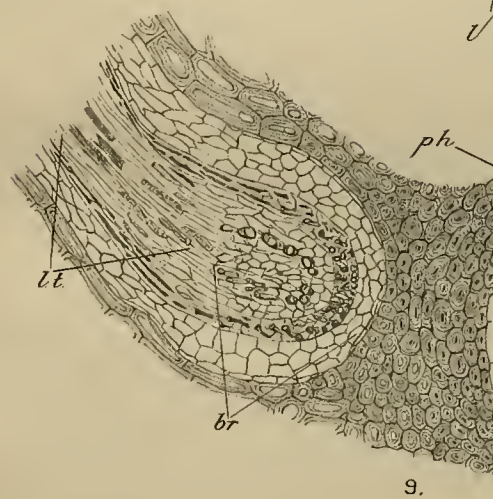
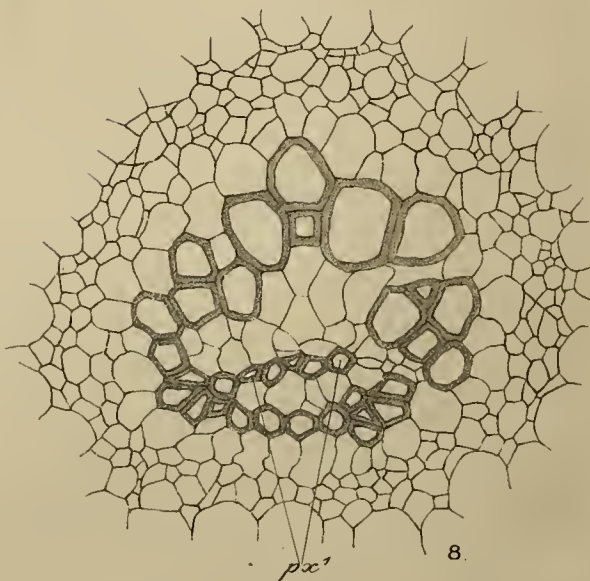
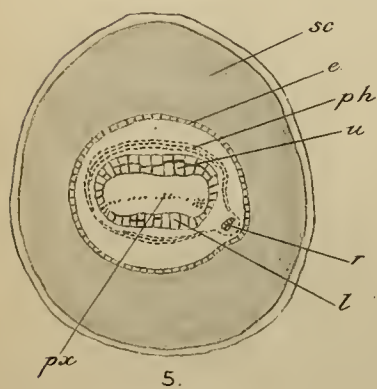
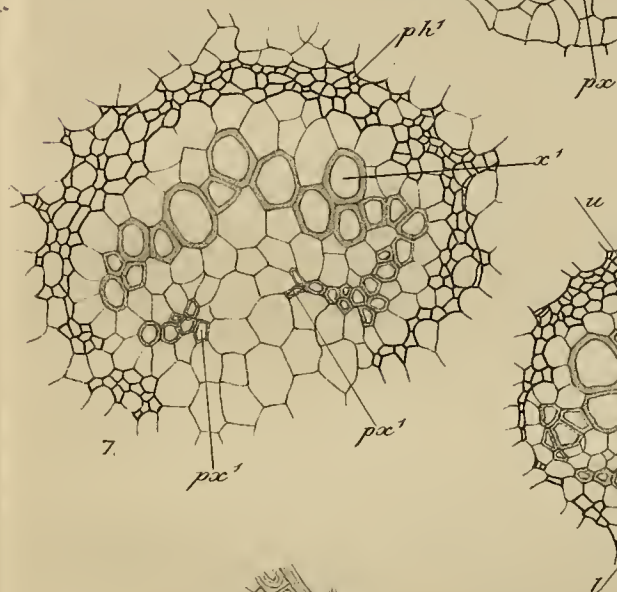
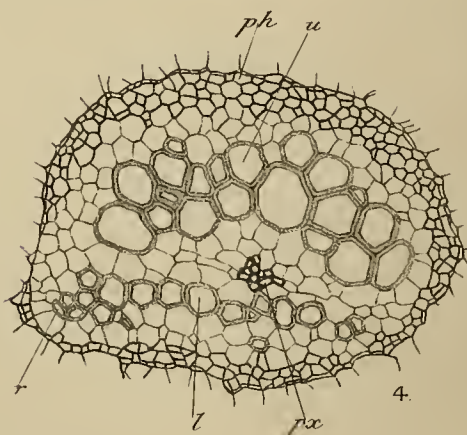
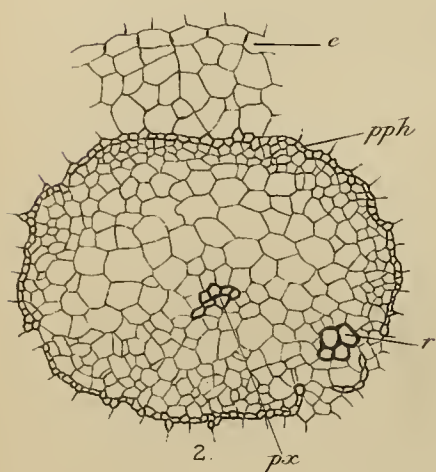
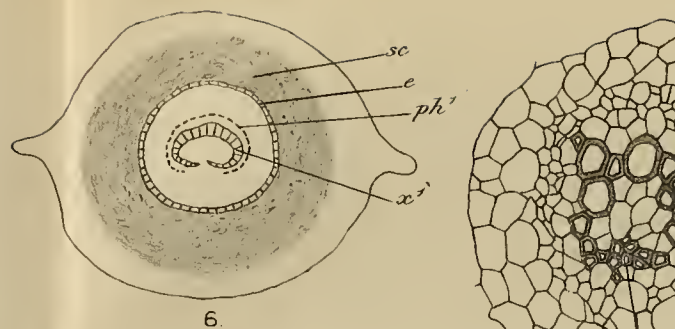
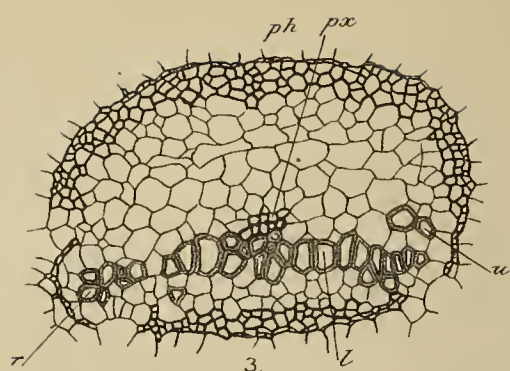
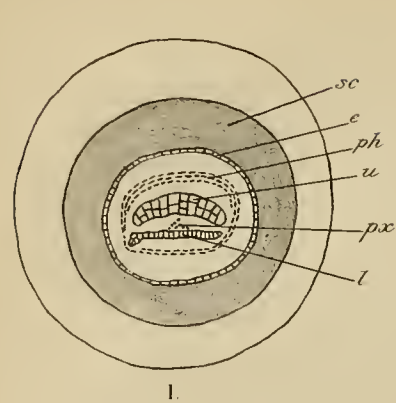


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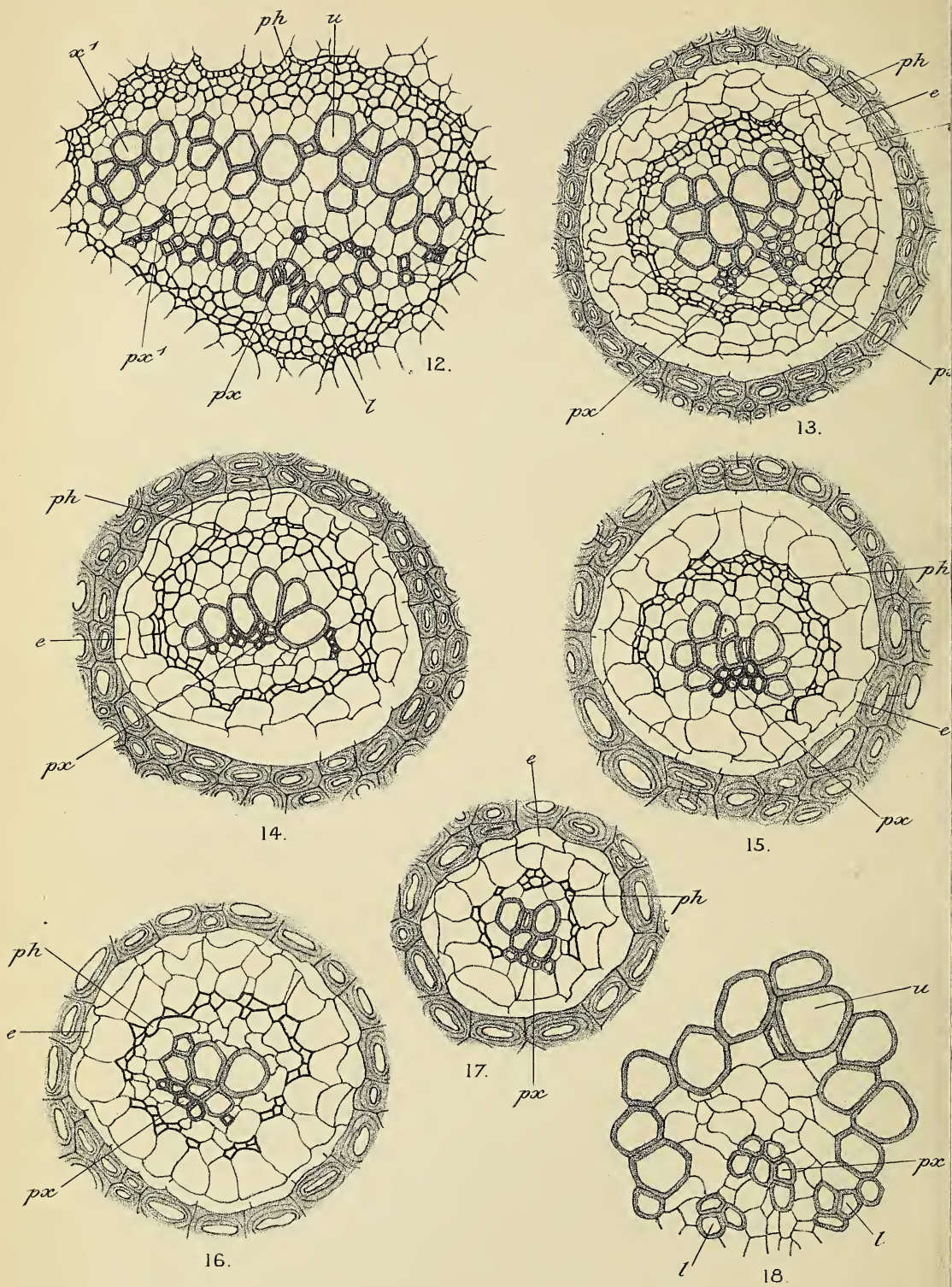




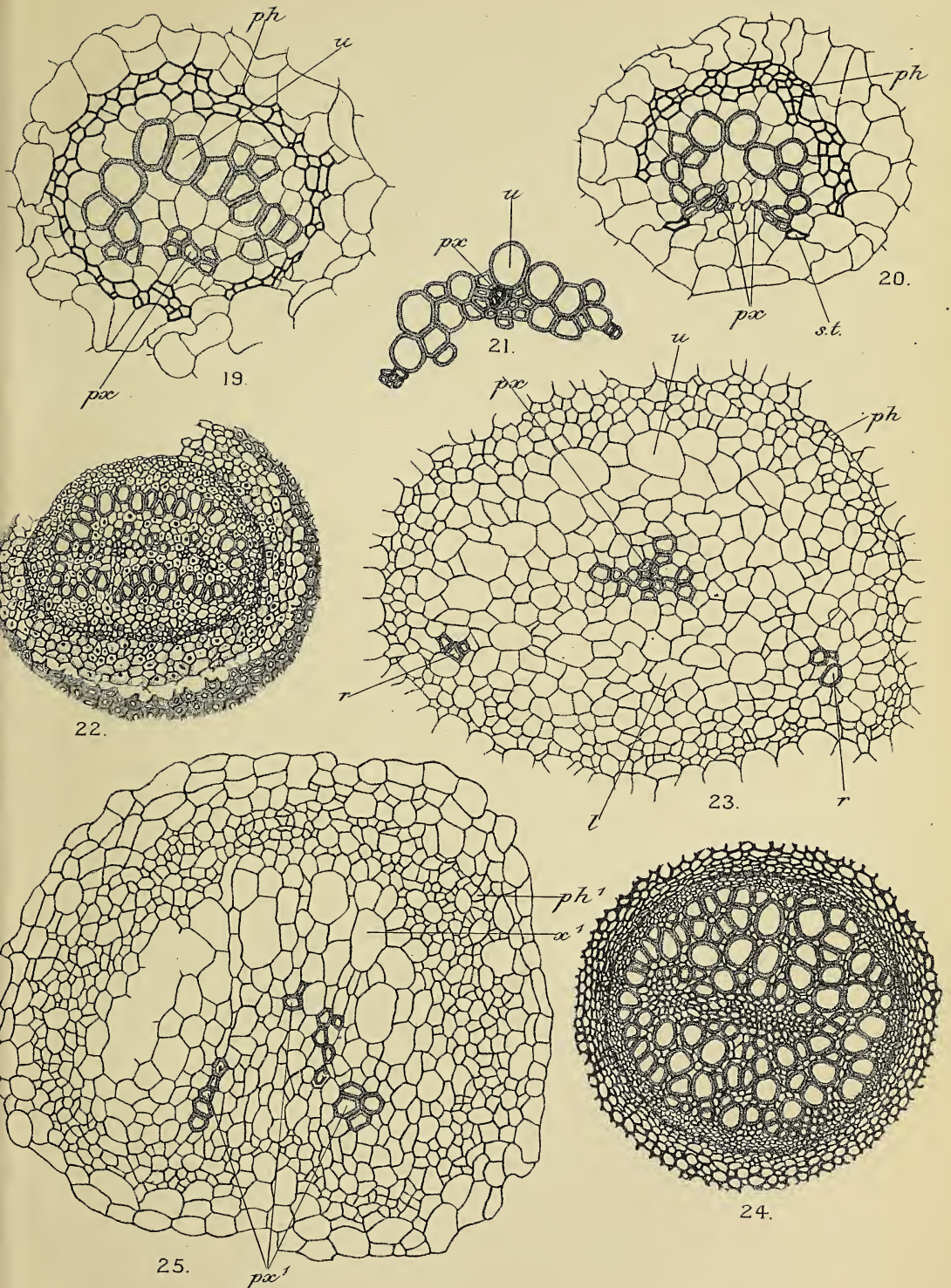






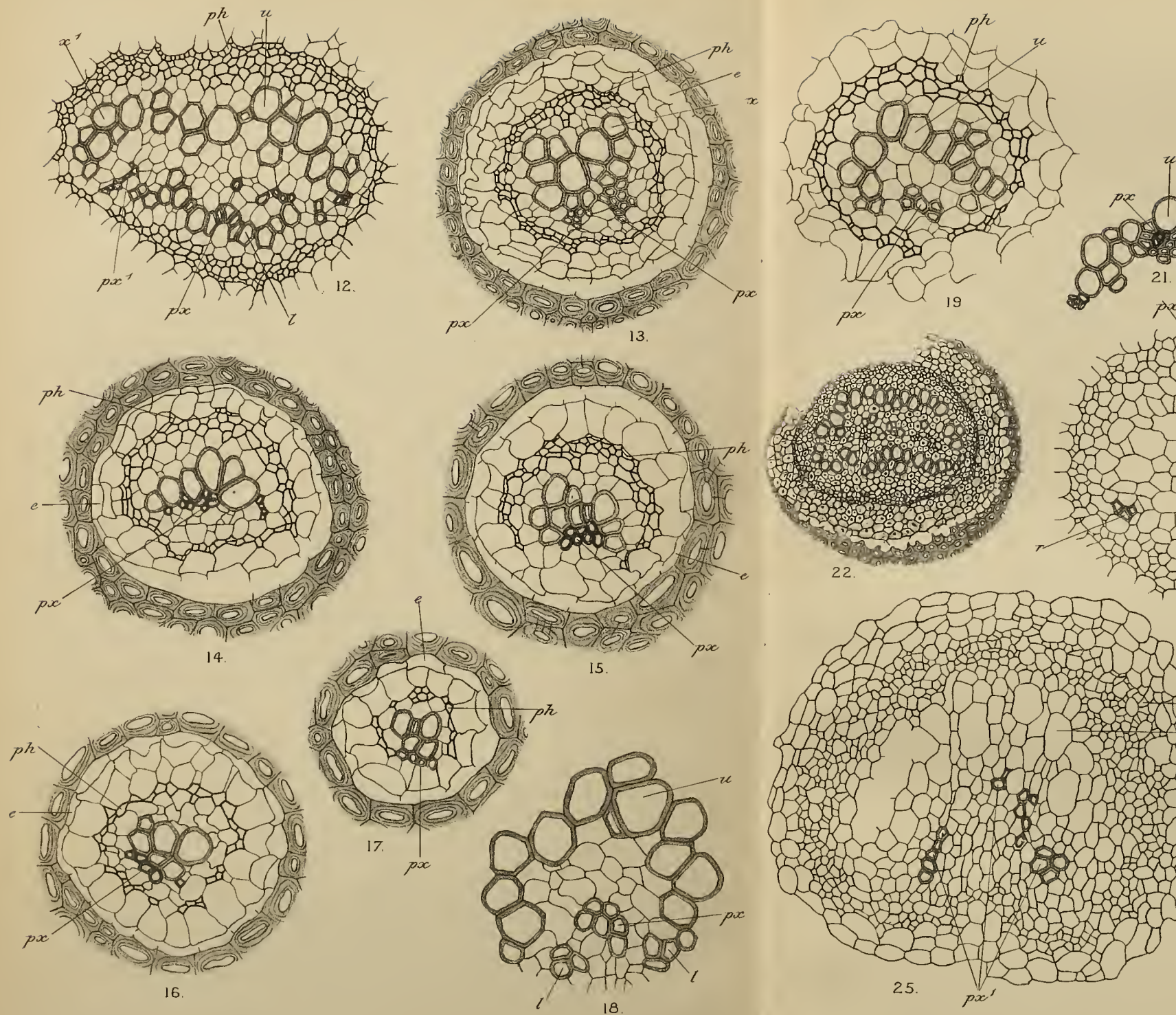










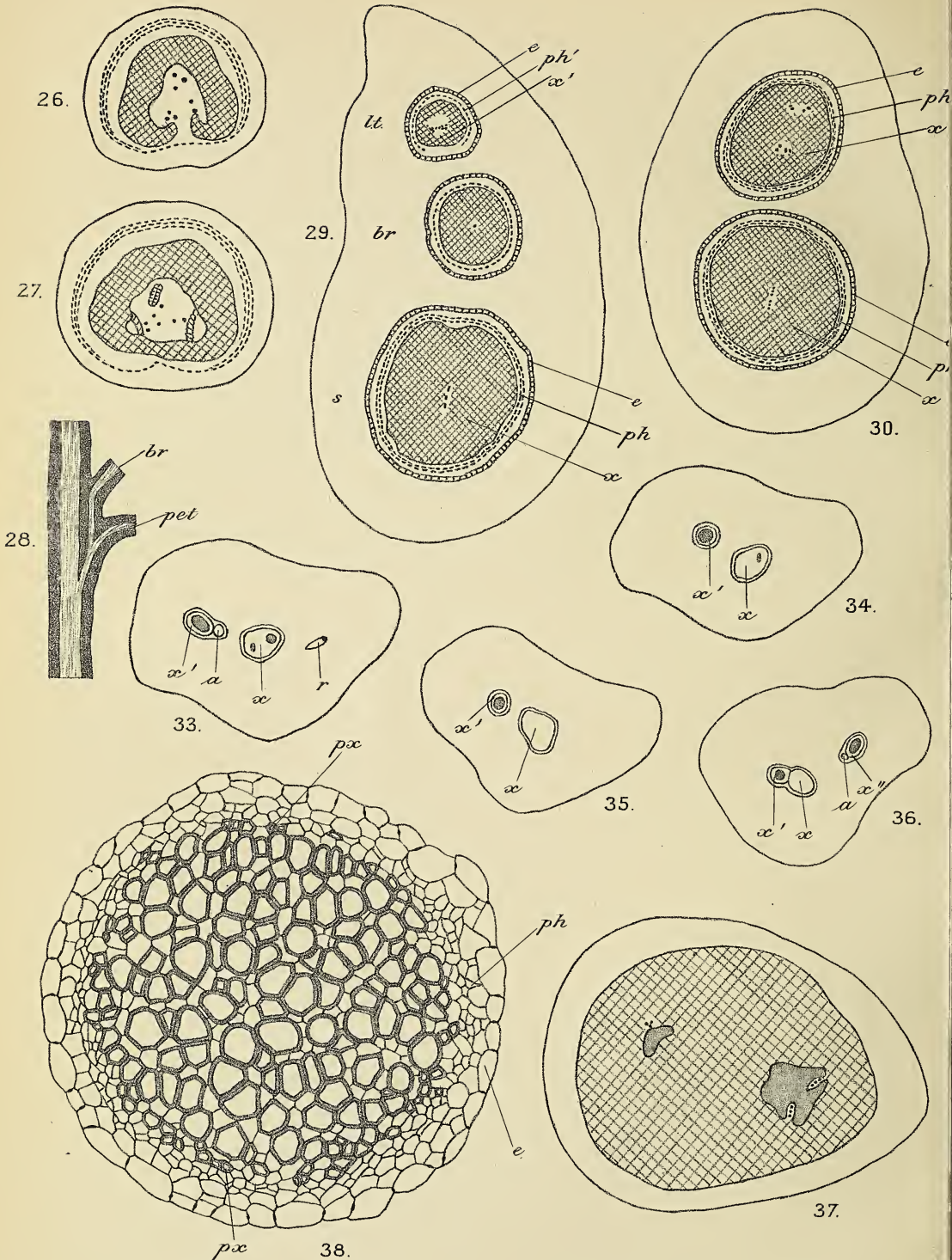


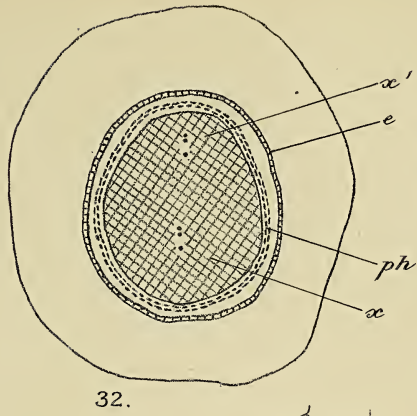
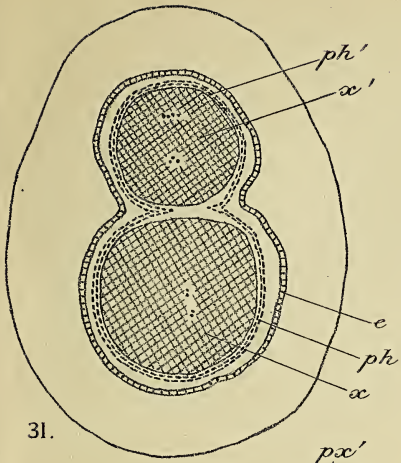
BOODLE. — ANATOMY OF HYMENOPHYLLACEAE.



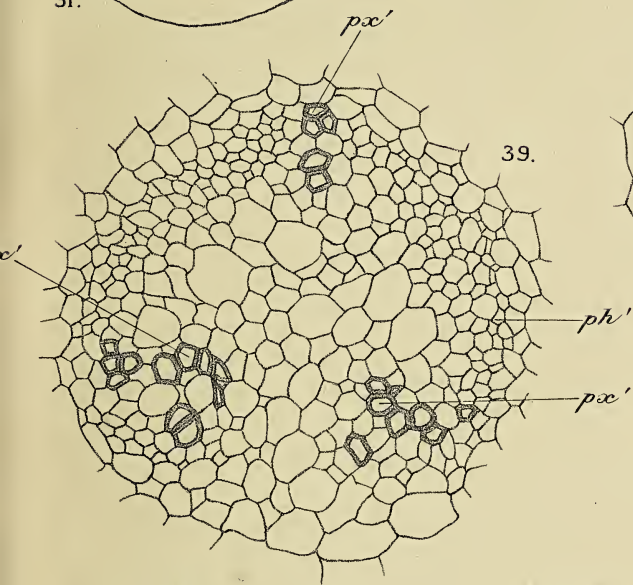




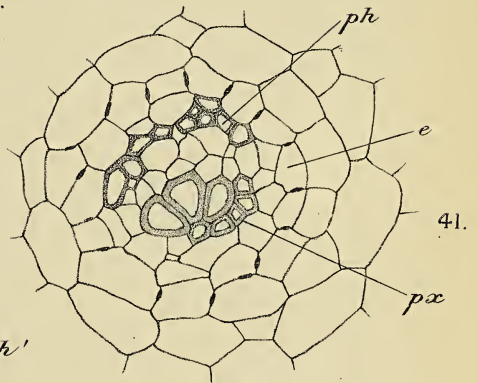




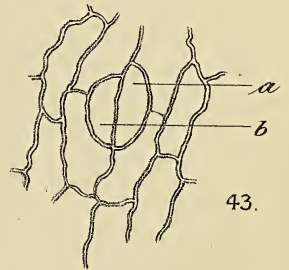
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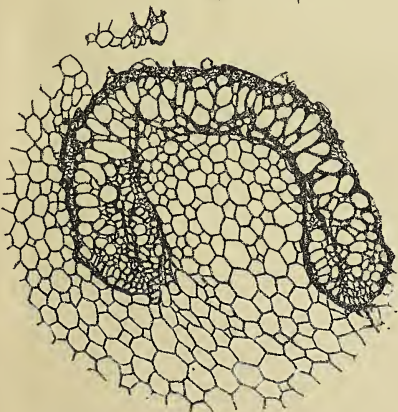
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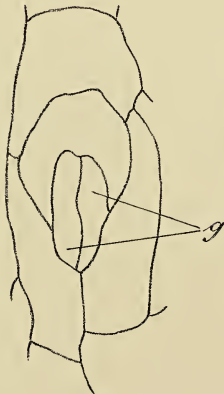
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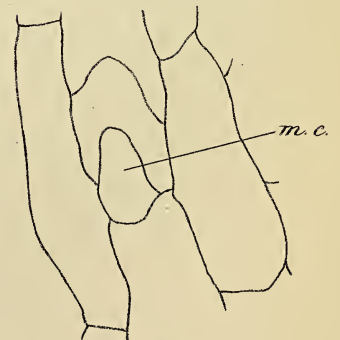
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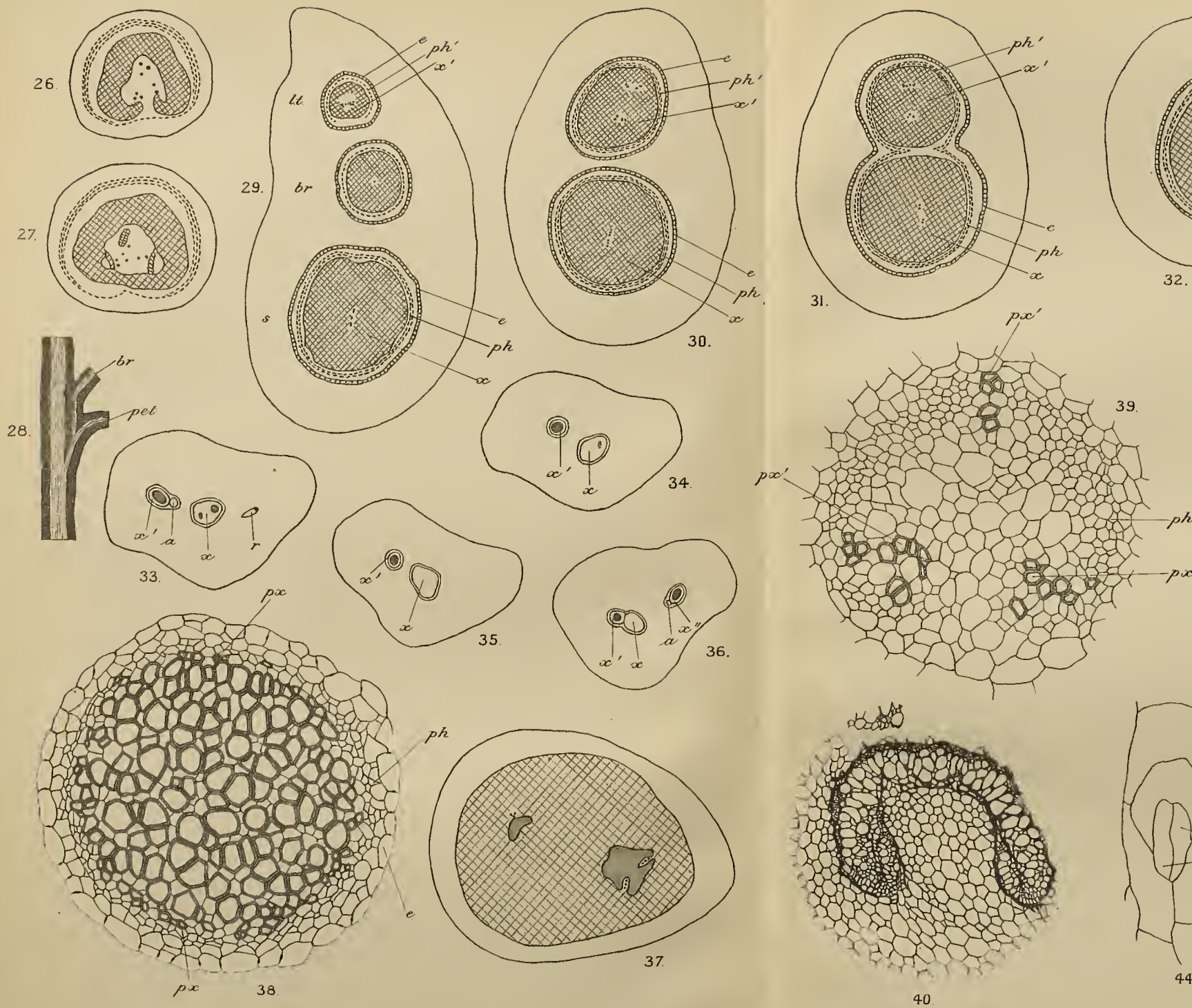
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BOODLE — ANATOMY OF HYMENOPHYLLACEAE.



# On the Structure of the Stem of *Angiopteris evecta*.

BY

R. F. SHOVE,

*Girton College, Cambridge.*



With Plates XXVIII and XXIX.



## INTRODUCTION.

THE Eusporangiate Ferns of the present day arouse particular interest, in that they are the living representatives of old Fern types only known to us in the fossil state. Any further knowledge of the structure of these Ferns is therefore of much importance to the palaeobotanist, and particularly is this so in the case of the Marattiaceae, once widespread and abundant, though now only represented by a few genera.

These considerations led to the following account of the anatomy of *Angiopteris evecta*, Hoffm., which is the result of work on a large stem brought from Ceylon by Mr. Pearson, of Kew, and generously placed at the disposal of Mr. Seward for investigation. In the examination of the roots some of the material used was obtained from the Cambridge Botanic Garden.

The account deals mainly with the anatomy of the stem, together with a few points of interest in the structure of the root. The arrangement and structure of the vascular tissue

[Annals of Botany, Vol. XIV. No. LV. September, 1900.]



in the leaf-bases and stipules have been examined, but only to a small extent, as work in this direction is being done by Mr. Brebner, of the University College, Bristol.

I should like here to express my hearty thanks to Mr. Seward, of Emmanuel College, Cambridge, for supplying me with the material upon which the following account is based, and also for his ready help and kindly interest throughout my work.

#### PREVIOUS WORK ON THE ANATOMY OF ANGIOPTERIS.

The first important account of work on *Angiopteris* occurs in the 'Monographie des Marattiacées,' by De Vriese and Harting<sup>1</sup>. In this paper several facts are stated in detail, but there is little investigation into the actual course of the vascular strands. The mistake of Harting's in confusing roots traversing the stem with stem-bundles is too well known to be more than mentioned.

In 1863 appeared the important paper by Mettenius, 'Ueber den Bau von Angiopteris.' Mettenius investigated a stem of *Angiopteris evecta*, Hoffm., but unfortunately the stem was decayed at the base, and had shown at the apex no signs of life for fifteen months<sup>2</sup>, facts which must be taken into consideration in comparing his results with those of later workers.

In this paper the course of the bundles is worked out in great detail; briefly stated, the vascular tissues are considered to form funnel-shaped zones with their lower ends in the axis of the stem, and their higher parts continued out into the leaves. A transverse section of these funnels would give rise to the rings of scattered bundles such as are seen in actual sections of the stem.

Segments from the outermost zone pass off into the leaves, and the gaps thus made are filled up by corresponding segments from the next inner zone. This method of compensation goes on from zone to zone. Mettenius further

<sup>1</sup> De Vriese and Harting ('53).

<sup>2</sup> Mettenius ('64), p. 504.

states that a portion of the second zone filling up the gap in the first, due to the presence of a leaf, unites with the segment of the first zone going to the leaf, so that at the point of insertion of the leaf we find anastomoses between the two outermost zones<sup>1</sup>.

Mettenius refers briefly to the structure of the stele in the stem and in the root, and worthy of note is the fact that he finds the protoxylem of the steles of the stem either central, or situated at the periphery of the wood nearest the organic axis.

In a paper by Russow<sup>2</sup> in 1872, there is no investigation into the course of the bundles, but the much debated question of the structure of the root-apex first comes under discussion.

Russow describes two kinds of roots: unbranched air-roots with twelve to twenty groups of protoxylem, the outermost elements only being lignified, and branched earth-roots with five groups, the elements of which are lignified to the centre.

According to Russow apical growth in the roots takes place by means of several cells; these are twelve to eighteen in number, prismatic in shape, proportionally large, and rich in protoplasm. The outer apical cells give rise to the cortex and epidermis; the middle apical cells form the axial vascular strands and root-cap tissues.

Longitudinal division occurs at intervals, and the cells thus formed take the place of those which have divided to form the cortex. The weaker earth-roots have a similar method of growth, but the number of apical cells present is smaller.

In connexion with Russow's paper it should be mentioned that the figure he gives of the apical cells in the root of *Angiopteris evecta* really represents the arrangement of the cells in the root-cap<sup>3</sup>.

In a paper by Kny<sup>4</sup>, contemporaneous with that of Russow,

<sup>1</sup> Mettenius ('64), p. 510.

<sup>2</sup> Russow ('72).

<sup>3</sup> Russow ('72), Taf. VIII, Fig. 161.

<sup>4</sup> Kny ('72).

it is stated that the roots of the Marattiaceae have one large apical cell, which is surrounded by numerous segments; these are similar in form and function to the apical cell, but show no transverse divisions.

This view of the apical cells is different from that of Russow, and is elaborated by Holle<sup>1</sup> in a paper published in 1875. Holle recognizes three forms of apical growth: firstly, the subterranean roots have a four-sided apical cell, from which the neighbouring cells can be clearly seen to arise; secondly, somewhat thicker roots have an irregular apical cell, between which and the neighbouring cells no hard distinction can be drawn; and, thirdly, the large aerial roots have no apical cell, but growth occurs by numerous initial cells in the manner described by Russow.

In a paper by Schwendener<sup>2</sup> in 1880 we find the view that a median section of the root-tip shows only two apical cells, and hence that apical growth is carried on by only four initial cells.

Bower<sup>3</sup> confirms this statement, and further says he has traced in a branch-root the development of four initial cells from a single cell of the endodermis.

The most important paper on the root-apex is that by Koch, 'Ueber Bau und Wachstum der Wurzelspitze von Angiopteris evecta, Hoffm.'<sup>4</sup>

The view here expressed is that no persistent apical cell is present at all, but that one of four particularly large cells assumes the function for a time. It becomes pressed into the organic centre of the root, and divides into four; three of these go on dividing and form meristematic tissue, but the fourth increases in size without division, and, being by pressure made to occupy a central position, repeats the process just described.

This method of growth occurs in small earth-roots; in the thicker earth-roots and in the large air-roots the above-mentioned four-celled complex indeed exists in the extreme

<sup>1</sup> Holle ('75).

<sup>2</sup> Schwendener ('80).

<sup>3</sup> Bower ('85).

<sup>4</sup> Koch ('95).



apical region, but is not necessary for the production of the root tissues. This is shown by the fact that at some little distance from the root-tip a gap, due to the disorganization of the constituent cells, appears in the midst of the cell complex, and growth is carried on by small meristematic cells which surround the cavity.

Turning now from the consideration of literature dealing exclusively with the root, we find an account by De Bary of a young stem of *Angiopteris evecta*. This showed a typical bundle-tube with wide foliar gaps, from the lateral margins of which two steles arise, ascend obliquely through the cortex, at the same time undergoing division, and then pass off into the leaves.

Particular points of interest, such as the structure and origin of the mucilage canals, and of the pits, or 'lenticels,' on the leaf-bases and stipules, are discussed in papers by Brebner<sup>1</sup>, Potonié<sup>2</sup>, Hannig<sup>3</sup>, and others. The curious intercellular network of rodlets, due to outgrowths from the walls of the cells, is characteristic of the parenchymatous tissues of the Marattiaceae, and is described by Luerssen<sup>4</sup>, Schenck<sup>5</sup>, Poirault<sup>6</sup>, and Kühn<sup>7</sup>.

#### EXTERNAL FEATURES.

In a description of the external features of *Angiopteris evecta*, no strict line of demarcation can be drawn between the stem proper and the massive leaf-bases with which it is completely covered.

The axis, which is obliquely ascending, ends at the base in a naked blunt cone (Fig. 1), but this region shows undoubted marks of erosion, and it is probable that it was more or less covered with leaf-bases similar to those found higher up on the stem. After the removal of the leaves the general form of the stem is seen to be obconical, and in its older parts distinct dorsiventrality of structure is presented.

<sup>1</sup> Brebner ('95).

<sup>2</sup> Potonié ('81).

<sup>3</sup> Hannig ('98).

<sup>4</sup> Luerssen ('73).

<sup>5</sup> Schenck ('86).

<sup>6</sup> Poirault ('93).

<sup>7</sup> Kühn ('89).

The upper surface of the naked cone that forms the base of the stem is brown and rough, and bears at intervals fragments of vascular tissue that have been exposed by the wearing away of the softer parenchymatous tissues. The under side, however, shows traces of an original surface, and bears several circular pits (Fig. 1, R), which have their origin in the decay of the tissues of the roots.

The stem is about 25 cms. in length, reckoning from the tip of the bare base to the summit of the smallest leaves visible from the exterior. In its widest part, that is, nearest the apex, it reaches a diameter of about 8 cms., and from this point it diminishes in diameter to about 1 cm. at the base.

The upper surface of the stem is bare for 3 cms. from the lower conical end, but here the first leaf (Fig. 1, A) occurs, somewhat shapeless owing to the wearing away of the tissues. From this point onwards no free surface is exposed on the upper side of the stem, but leaf-bases, closely wedged together, increase in number and size. The shapeless character of the lower leaf-bases is clearly seen in Fig. 1.

The lower surface of the stem bears no leaves for a distance of 8 cms. from the base, but is densely covered with roots. These emerge directly from the free part of the stem, and, as will be seen later, the arrangement of the vascular network is modified in this region to facilitate their passage to the exterior. The roots occur for the most part in a definite region on the lower surface of the stem, which bears also one small leaf-base. This leaf-base, together with the lowest of those occurring higher up, is penetrated in every direction by roots, so as to almost obliterate the tissues of the leaf-base proper. Further up the stem the roots occur singly, making their way to the exterior either between or through the leaf-bases. Correlated with this diminished production of roots is a tendency on the part of the stem to assume a radial rather than a dorsiventral structure.

Owing to the incomplete development of the younger parts of the stem, it could not be determined with absolute certainty whether a radial structure is present in the plant during the

later years of its growth. However, from the manner of grouping of the young leaves round the apex, this appears to be the case.

The roots vary in length from 14 to 50 cms. with a diameter of about 1 cm. ; they are for the most part unbranched, but some bear a few weak branch-roots at long intervals. They are cylindrical in form, with a black wrinkled surface due to the drying up of the tissues, and correspond in structure to the earth-roots of Russow, possessing, however, in general more than five groups of protoxylem.

The apex of the stem is protected by the circinately coiled fronds of the young leaves, each leaf being surmounted by those older than itself.

Turning now to the consideration of the leaves, we find that these occur in the older parts of the stem, mainly on the upper surface, but a few small ones are also found on the under surface, half concealed by numerous roots.

In the younger parts the leaves are arranged round the axis with an approximation to radial symmetry.

The oldest leaf-bases are more or less shapeless masses, owing to decay, but higher up on the stem the leaves display a well-marked differentiation into leaf-base and stipules. In the lower parts of the leaf-base, the petiole and stipules are confluent into one swollen mass, which is united to the stem by a narrow base. This narrow lower region widens out rapidly into a mass which is convex towards the exterior and plane towards the axis of the stem. The leaf-bases, however, vary considerably in size and in method of insertion on the stem, and often, particularly in the smaller leaves, the above-mentioned dorsiventrality of structure is not obvious.

The stipules become separate from the leaf-base proper towards the axis of the stem, and enclose a space, in which the young frond is coiled. The partition joining the stipules is concave inwards, and closely pressed against it is the convex outer surface of a younger leaf. Figs. 3 and 4 show the coiled petiole of the leaf with its enveloping stipules seen in longitudinal and transverse view.



In Fig. 3, a longitudinal section taken a little to one side of the middle line of the leaf, the outermost stipule (s) is continuous with the tissues of the leaf-base. The wrapping of one stipule (s) over the other (s') is seen also in Fig. 4, which is a transverse section of the leaf shown in Fig. 3 taken along the line AB. The space between the stipules is filled up with ramenta.

The leaves attain their maximum size about the middle of the stem; here the cicatrix left by the fallen petiole measures from 5 to 6 cms. in diameter. Even among these, however, a small leaf-base not infrequently occurs.

The surface of the stipules and of the swollen leaf-base is covered with numerous scars (Fig. 1, L), somewhat lighter in colour than the other peripheral tissues and slightly depressed below the surface. The scars are oval in outline on the stipules and the sides of the leaf-base, but circular on the convex rounded portion; on the petiole itself they form narrow short streaks or spots. These pits, the so-called lenticels, are small on the petiole, but may attain a diameter of from 1 to  $1\frac{1}{2}$  cms. on the leaf-base.

In the older leaves the surface of separation of the petiole from the leaf-base is clean-cut, and in it the ends of the steles that have been snapped asunder by the breaking away of the petiole are clearly seen, arranged more or less in circles.

In the younger leaves the lower part of the petiole still remains attached to the base, and its upper end is jagged and broken, where the rest of the leaf was artificially removed.

Finally, in the youngest leaves the petiole still remains circinnately coiled, and is completely enclosed by the two overlapping stipules, which are covered with numerous ramenta.

These ramenta are not found on the old leaf-bases, the surface of which is irregularly corrugated, firm in texture, and brownish-black in colour.

VASCULAR SYSTEM IN THE STEM.

In the determination of the course of the vascular strands in the stem of *A. evecta*, two methods of working were found most successful.

The comparison of successive transverse sections in the first place gave information as to the origin of the leaf-trace bundles, the method of compensation from zone to zone, and the origin and passage to the exterior of the roots.

A clear conception of the arrangement of the vascular tissues into a network could, however, only be gained by the removal of the parenchymatous tissue and the exposure of the strands.

Softening the tissues by boiling in potash was found inexpedient, as, though the labour of removing the parenchyma was thereby much diminished, the vascular strands became too weak to maintain their relative position. It was found most satisfactory to pick away the parenchyma with a sharp knife, and thus expose the vascular tissue.

In accordance with the dorsiventral structure of the stem as seen from the exterior, the vascular network was found to be different in character on the two sides of the stem. This is seen at once by comparing Figs. 5 and 6, which represent the superficial vascular tissue on the upper and under side of the stem respectively. In Fig. 5 strands from more deeply lying vascular zones are also represented.

On the upper surface of the stem the meshes of the network are small and more or less square in outline; the strands destined for the leaves form obliquely ascending portions of the vascular framework, which stand one above another at short intervals. If the dissected stem, shown in surface-view in Fig. 5, is seen from the side, the strands bending out into the leaves appear as projecting and curved bracket-like portions of the stellar lattice-work.

In Figs. 5 and 6 the strands belonging to the same leaf have the same lettering; thus in Fig. 5, the bundles marked

*e* belong to leaf I, the bundles marked *c* to leaf IV, and so on. The sets of leaf-trace bundles are arranged in an irregular spiral round the stem, but the actual phyllotaxis could not be determined owing to the apparent discontinuity of the spiral on the under side of the stem.

Only a few roots are produced by the vascular strands occupying the upper surface of the stem; these come sometimes from the outermost, sometimes from an inner zone.

In Fig. 5 only five roots (R) have been shown, since the position of the remainder was uncertain owing to their removal during the dissection of the stem, but probably about double the number given in the drawing should have been represented.

On the lower surface of the stem the meshes of the network are much drawn out, and there are few anastomoses between the strands; further, the step-like arrangement of the leaf-bundles as seen in the upper side of the stem is here missing, and the strands going to the leaves are much more parallel with the outermost zone.

The whole tissue of the under side of the stem is thickly penetrated with roots, as is seen in Fig. 6; the circular outline of the roots indicates their passage outwards in a direction perpendicular to the longitudinal axis of the plant. The sections of the roots represented in the drawing do not all lie in the same plane, but those in the neighbourhood of the shaded bundles are nearest the axis of the stem, as here the parenchymatous tissue has been removed to the greatest extent. The shaded strands are those belonging to the second vascular zone.

The roots originate, a few in the outer, the majority in the inner zones, but some actually arise from stelar tissue nearer the upper than the under side of the stem, and from there make their way to the lower surface through almost the whole thickness of the stem.

The roots emerge in general at right angles to the surface, or less frequently in an obliquely descending direction. The



roots represented in Fig. 1 have an upward direction, but this is due to the action of an artificial pressure.

We will turn now to the consideration of the leaf-trace bundles. These come entirely from the outermost zone of vascular tissue, and in this point the stem now described appears to differ from that examined by Mettenius, who clearly states that in his plant the leaves are supplied with strands coming from the second as well as from the outer zone<sup>1</sup>.

As can be seen from Figs. 5 and 6, the number and arrangement of leaf-strands vary considerably<sup>1</sup>; it would perhaps convey the truest idea of the arrangement to say that a meshed segment of the outermost zone passes off into a leaf. The difference in character of the vascular tissue on the under and upper side of the stem necessitates a different arrangement of the leaf-trace bundles in the two cases. The meshes formed by the strands in the leaf-bases on the under side of the stem are long and narrow, and in consequence of this the strands themselves do not anastomose so frequently. In Fig. 6 leaf A may be taken as typical of this arrangement, and in Fig. 5 leaf I represents the most general form of the leaf-trace bundles on the upper side of the stem.

Leaving for the future the more detailed account of the foliar strands, we turn to the consideration of the vascular tissue in the inner zones of the stem.

The gap in the outermost zone occasioned by the departure of the leaf-trace bundles is filled up by a segment coming from the next inner zone. This is shown in Fig. 5, where the gap in the superficial zone of vascular tissue, formed by the departure of four strands to leaf I, is filled up by a meshed segment rising from the second zone. The gradual passage outwards of this compensating segment from the inner to the outer zone is shown, in the drawing, by a diminution in the degree of shading; the points of union of the two zones are shown at A, B, C, D.

The compensating segment unites right and left of the leaf-base with the outermost zone, and, after continuing for

<sup>1</sup> Mettenius ('64), p. 512.

a short time in the superficial zone, takes part in the formation of strands for leaves II, III, IV, V. The gap thus caused in the second zone is filled up in a similar way by a segment coming from the third zone. In Fig. 5 the dotted lines show the strands of the third zone; a portion of this passes into the second zone opposite leaf IV at E, F, G, H, and is here darkly shaded. Still continuing its course outwards, the segment passes into the superficial zone at M, N, and fills up the gap caused by the giving off of strands to leaf IV, and finally shares in the formation of strands destined for leaves V, VI, and VII. It probably shares also in the formation of a fourth leaf, not shown in the drawing, but indicated by the direction of the strand K.

Compensation is thus carried on from zone to zone in the way described by Mettenius, who worked out the relative arrangement of the compensating segments in great detail. The regularity described by him is, however, not apparent in the stem now described.

Fig. 6 shows the compensating segments from the second zone filling the gap in the first, but the large number of the roots and the irregular arrangement of the leaves make it impossible to trace the compensation to greater depths. As in Fig. 5, the shaded strands are those belonging to the second zone.

In considering the foliar strands we find that at some level a transverse section of a leaf-base shows from four to six steles arranged along the convex side of the leaf-base, so that between them and the flattened side of the leaf there is a considerable mass of parenchyma. The strands of the meshed segment corresponding to these steles as seen in transverse section branch and anastomose freely, and soon assume the arrangement of a meshed cylindrical surface. Further anastomoses and divisions then take place, resulting in the formation of the fine stipular threads, and of the concentric zones occupying the higher part of the leaf-base.

Figs. 9-12 show a series of transverse sections of a leaf-base. At its point of junction with the stem the leaf shows

six strands arranged as in Fig. 9; these divide and give off branches from their inner surface which turn towards the flat side of the leaf, so that at a distance of about 2 cms. from the position of the first section, they represent the appearance shown in Fig. 12.

The initial strands are in general of the same size; the two large side strands described and drawn by Mettenius<sup>1</sup> do not appear to be present.

The series of transverse sections shown in Figs. 9-12 corresponds to leaf I in Fig. 6; this particular leaf was one of the largest on the stem.

In Figs. 7 and 8 is shown the actual network in the leaves formed by the branching strands; Fig. 7 shows the vascular tissue of a leaf from the dorsal, Fig. 8 those of a leaf from the ventral side of the stem; in both cases the strands had not attained their full development. The cut ends of the steles lie in the same plane, being exposed in their present position when the corresponding leaf-base was cut from the stem; in Fig. 7, however, the strands A and B belong to other leaf-bases. Fig. 8 shows a root (R) originating at the base of a leaf-trace bundle. The different character of the meshes of the network in the leaf according to its position on the stem is clearly seen in the drawings.

It is interesting to compare the course of the vascular strands in *A. evecta* with that in *Kaulfussia* and *Marattia* described by Kühn<sup>2</sup>.

*Kaulfussia* possesses a single-meshed cylinder of vascular tissue enclosing a central strand; from the outer zone segments are given off to the leaves, and compensation for these is afforded by segments from the central strand.

The vascular tissue in *Marattia* is more complicated; here there is a central strand enclosed by two meshed zones, from the outermost of which segments are given off to the leaves, while a compensating segment from the second zone fills up the gap in the first, and a branch from the central strand fills up the gap in the second. *Angiopteris* differs from

<sup>1</sup> Mettenius ('64), p. 512.

<sup>2</sup> Kühn ('89).



*Marattia* only in having from four to five zones instead of two ; the plan of compensation from zone to zone is identical.

The general scheme of the arrangement of the vascular tissue in *Angiopteris* is most clearly conceived by considering it in connexion with the insertion of the leaves. The leaf-bases, which are set in a rough spiral on the stem, show in their lower parts a meshed segment of vascular tissue having the form of part of the surface of a cylinder. This segment passes from the leaf-base into the outermost zone of the stem, uniting right and left with the strands of this zone. Then continuing in an obliquely descending direction, it passes on into the second zone, and so on until it reaches the longitudinal axis of the stem, where it unites with other former leaf-trace bundles and loses all individuality.

Since the leaves are set so thickly on the stem, there is very little vascular tissue that can be definitely stated to belong to the outermost zone ; in fact, any part of the outer vascular tissue is more truly considered as forming the lower strands of the leaf-bundles on their way to the interior, than as a portion of a definite meshed cylinder.

A transverse section of the extreme tip of the base of the stem (Fig. 13) shows one large central stele (S) ; this, at a distance of about half a centimetre from the end, bifurcates, and by further division of the strands thus produced an irregular network arises. It should, however, be remembered that the base of the stem has suffered an unknown amount of denudation, hence the vascular tissue here described forms only part of the original system.

Figs. 13-18 show a series of transverse sections of the stem taken at distances of about half a centimetre.

The steles are seen to undergo frequent division, and are scattered irregularly through the section ; only towards the end of the series does the grouping into concentric circles make itself apparent. Numerous roots (R) are seen to traverse the stem, but their places of origin are, with a few exceptions, not shown, owing to their position higher up in the stem. It should be noted that the roots reach the periphery of the

section on the left side, which corresponds to the under surface of the stem. The circular outline of the roots at any point indicates their perpendicular descent through the tissues of the stem at this particular part of their course. In Fig. 14 partly decayed steles are shown making their appearance at the periphery of the root; these assume positions further removed from the surface of the stem as the diameter of the sections increases, and are obviously the remaining parts of an outer meshed cylinder of vascular tissue.

The steles shaded in the diagrams are those arising from the branching of the single large stele (s) shown in Fig. 13.

The denudation of the stem has apparently taken place to a greater extent on the upper surface, since it is there that these decayed steles make their appearance in the greatest numbers. Moreover, on the under part of the stem there are traces of an original surface.

Towards the apex of the stem the vascular system maintains the same general characters as in the fully mature parts, and, though on a much smaller scale, a transverse section of the stem at a distance of about 1 cm. below the apex is closely analogous to those taken at levels of complete development. The closing up towards the apex of the meshes in the vascular tissue, and the change of the outer network into a closed cylinder, which are described by Mettenius<sup>1</sup> in his paper on *A. evecta*, are probably to be correlated with the decay in the upper parts of the stem.

By carefully removing the young leaves from the apex of the stem, and noting the position of the leaf-trace bundles, the manner in which these steles make up the roughly concentric circles shown in a transverse section of the stem can be clearly seen.

With regard to the development of the roots from the stem-bundles, there appears to be no regularity of arrangement; some leaf-bases contain no roots at all, while others are so much penetrated by roots making their way to the

<sup>1</sup> Mettenius ('64), p. 506.

exterior as to show an almost complete absence of parenchymatous tissue. The roots in general originate at points where the steles anastomose; this is so in the case of the roots shown in Fig. 5.

## HISTOLOGICAL STRUCTURE.

### I. Stem.

A transverse section of the stem of *Angiopteris* shows the vascular bundles grouped in roughly concentric circles; the arrangement has been described and figured by Mettenius<sup>1</sup>. The outline of the stem-steles, as seen in transverse section, varies from roughly circular to strap-shaped, the latter arising by the union of two or more of the former.

Union takes place between steles lying right and left of each other, and not between those lying approximately on the same radius, except in connexion with the giving off of leaf-trace bundles or compensating strands. The longer diameter of the strap-shaped steles varies from 15 to 3 mm.; the more or less circular steles measure from 5 to 2 mm. across.

A transverse section of the stem at any level does not cut all the steles transversely, owing to the oblique direction of their course; hence, to determine the position of the protoxylem for a whole section, it is necessary to isolate each stele and to cut it separately. As a result of such procedure it was found that the steles are both mesarch and endarch in structure. Fig. 2 shows the position of the protoxylem groups for a complete transverse section of the stem. The protoxylem elements are recognized in the mature steles by their small size and frequently crushed condition; they appear thus in Figs. 21 and 26.

The investigation of very young steles taken from the apical region of the stem confirms the results obtained from the consideration of the mature bundles. In these young

<sup>1</sup> Mettenius ('64), Taf. I, Fig. 1.



steles (Figs. 23 and 25) the protoxylem is found in groups of two or more spiral tracheides situated along the periphery and in the centre of the stele; the number of protoxylem-groups depends upon the size of the stele, the larger steles containing about five or six such groups.

The earliest protoxylem appears along the periphery of the stele; that is, no steles occur in which the central groups are unaccompanied by some at the periphery. The protophloem arises on the outer side of the stele in the form of a discontinuous arc of small, somewhat thick-walled elements (Fig. 23 PP) which, from the presence of minute granules on their walls, and from their method of attachment to each other, appear to be small sieve-tubes. The position of the protophloem is shown diagrammatically in Fig. 2, and is present in Fig. 25 as a somewhat indistinct layer interior to the large sieve-tubes on the left side of the section.

In a young stele the protophloem and protoxylem are seen, roughly speaking, at the opposite ends of the same radius, the protophloem being towards the periphery of the stem. In very young steles, however, the arc of phloem is found differentiated before any protoxylem elements have been lignified, and this is particularly striking in the steles in the young leaf, where the sieve-tubes are of considerable size.

The arc of protophloem is never completed round the stele, but the next stage in the development of the tissues after the appearance of the protoxylem is the differentiation of large sieve-tubes exterior to the protophloem. A continuous ring of sieve-tubes is finally formed round the xylem, but in certain stages of development, for example in that represented in Fig. 23, the sieve-tubes exterior to the protophloem are larger and thicker-walled than those in the neighbourhood of the peripheral protoxylem.

The lignification of additional tracheides takes place at the same time as the formation of the ring of large sieve-tubes, and in the mature stele we find the concentric structure of the ordinary Fern-type. The ring of sieve-tubes is more con-

spicuous in the young than in the mature steles, as is seen by comparing Figs. 21 and 25.

The phloem is of greatest breadth on the side of the stele turned away from the axis of the stem, a fact that has been already noted by Mettenius and others. This is not very obvious in the stele shown in Fig. 21, owing to the crushing of the phloem on the outer side, but is seen best by comparing Figs. 19 and 28. Fig. 19 shows the edge of the stele in the neighbourhood of the protoxylem, and here the phloem-band is narrow. Fig. 28 is a drawing of the outer side of the stele, and here the phloem, made up of large sieve-tubes and protophloem elements, is of considerable width. The greater width of the phloem on the outer side is thus to be attributed to the presence here of the protophloem, which, as has been mentioned above, is never produced on the inner side of the stele.

The protophloem appears in the mature stele as a discontinuous arc of somewhat crushed elements (Fig. 28 PP).

The centrifugal method of development of the phloem in *Angiopteris* is contrary to that which prevails in other Ferns. Thus in the steles of the Gleicheniaceae, Polypodiaceae, and Cyatheaceae the protophloem occurs as a similar layer of small crushed elements, but the subsequent sieve-tubes are developed interior to these. In the genus *Matonia*<sup>1</sup>, the protophloem presents an appearance similar to that in *Angiopteris*, but here again the further development of the phloem is centripetal.

Further evidence in favour of the view that in *Angiopteris* the elements in question really constitute protophloem, lies in the fact that no crushed elements occur exterior to the large sieve-tubes, but that these border directly on the cells of the stem-parenchyma (Figs. 19 and 28).

In a transverse section of the younger parts of the stem the degree of development of the steles is proportional to their distance from the centre: thus a stele in the periphery of the section may have completely lignified xylem, another nearer

<sup>1</sup> Seward ('99), Fig. 34.

the centre may possess only isolated groups of spiral tracheides, while a third, nearest of all to the axis of the stem, shows only an arc of protophloem. The method of development of the stele can thus be followed in a single transverse section of the stem.

The concentric structure of the steles with the protoxylem arranged as just described is found in the steles occurring in the leaf-bases, the normal structure of the leaf-bundles only making its appearance where the swollen leaf-base passes over into the petiole proper.

As has been frequently stated by previous investigators, no endodermis is present round the stem steles.

The smallest elements of the protoxylem are spiral tracheides, but somewhat larger tracheides have a form of thickening transitional between spiral and scalariform, which may be described as reticulate. The spiral and reticulate tracheides occur both at the periphery and in the centre of the stele, and are seen in longitudinal section in Fig. 31.

The large sieve-tubes with their transverse walls are similar in structure to those described by Poirault<sup>1</sup> for the petiole and root.

As is well known, the stem of *Angiopteris* is destitute of sclerenchyma, though this is present in the petiole.

Investigations into the character of the actual apex of the stem have not been successful in obtaining any definite result. The stem apparently ends in a flattened or a very slightly convex surface, which is protected by the young leaves with their stipules. In the centre between the youngest leaves the apical tissues are visible as a small white spot, due to the large size of the cells in this region. The actual apex is occupied by several large cells, but the exact arrangement of these could not be determined owing to failure to obtain an accurately transverse section. These cells may be the product of division of an apical cell, or there may be several initial cells as is certainly the case in the apices of the leaves. The vascular strands can be traced up very near the position of these apical cells in the form of strands of small-celled meristem.

<sup>1</sup> Poirault ('94), pp. 139, 194.



## II. Root.

In the plant investigated the roots produced were all of one kind, and similar in structure to the earth-roots of *A. evecta* described by Russow. Transverse sections were cut of twenty-five roots taken at random from the plant, and in these the number of protoxylem-groups varied from ten to thirteen; further, the xylem elements were lignified to the centre of the root. The small number of protoxylem-groups and the total lignification of the xylem is described by Russow for earth-roots; besides these, however, he mentions large air-roots, in which from eighteen to twenty protoxylem-groups were present, and in which only the outermost elements of the xylem were lignified. These air-roots appear to be absent in the particular variety of *A. evecta* under examination, but investigation into the structure of the large aërial roots has been carried out on material from the Cambridge Botanic Garden.

After their entry into the ground the large roots branch frequently, more than one side-root being given off at times from the same level. The branch-roots bear no definite relation to the protoxylem-groups in the main root; thus the vascular tissue in the branch may be in connexion with one, two, three or more protoxylem-groups in the mother-root (Fig. 27).

The structure of the earth-roots has been given in detail by Harting and by Mettenius, hence it will be only necessary here to allude to one or two points.

Figs. 22 and 24 show the difference in structure between an earth- and an air-root. The endodermis is well marked in both, though it does not appear very clearly in the photographs, but its structure is better seen in Fig. 20, which is a drawing of a well-preserved root obtained from the Cambridge Botanic Garden. In a longitudinal section of the root the walls of the endodermal cells appear waved, this being due to the occurrence of alternate bars and pits

on the longitudinal radial walls. This structure has already been described by Mettenius<sup>1</sup>.

In the large air-roots the outer elements only of the xylem become lignified; the inner elements (Fig. 24 A) retain the form of tracheides, but their walls are not thickened in any way. In some cases the xylem of the air-roots was lignified half-way to the centre.

In one air-root examined, certain of the rays of unlignified tracheides were not completed at their outer ends by groups of lignified elements, but instead of these, groups of phloem occurred. In one ray there lay successively phloem, a lignified tracheid, and unlignified xylem-elements. The non-completion of the ray of unlignified tracheides is of interest, since Koch states that in the normal development of the root these more central tracheides are the first to be differentiated<sup>2</sup>, while what is generally regarded as protoxylem is formed later at the outer end of the rays. These small elements at the outer end of the ray are certainly the first to be lignified, whether or not the more central unlignified elements in the shape of tracheides are formed later.

The cortex in the root, as has already been described by Harting and Mettenius, is made up of two regions, the line of division between the two being particularly clear during the passage of the roots through the stem. The inner cells of the cortex bordering on the endodermis are thin-walled, rich in starch, and separated by intercellular spaces, which contain interwoven threads given off from the outer walls of the cells. These are the rodlets of Kühn and others. The cells of the outer cortex have gelatinous walls, no cell-contents, and are separated by no intercellular spaces.

The outermost cells of the cortex in the root are separated from the cortical cells of the stem by a layer of gelatinous substance, apparently similar in nature to that which constitutes the cell-walls in the outer cortex. In the older roots the distinction of the cortex into two layers is not so apparent, owing to the fact that the cells of the outer cortex

<sup>1</sup> Mettenius ('64), p. 518.

<sup>2</sup> Koch ('95), p. 377.

are crushed together to form a protective tissue. Among these crushed elements occur at intervals short lignified cells; these are thick-walled, with pointed ends, and probably function as idioblasts for mechanical support.

In two cases, probably anomalous but certainly of interest, the roots of *Angiopteris* showed dichotomous branching. The first case was that of a large air-root obtained from the Cambridge Botanic Garden; the two branch-roots produced by the dichotomy of this measured only about 1 cm. in length, hence their vascular elements had not been differentiated, and in fact the branches could be hardly said to constitute more than root-tips. The mother root was 1 cm. in diameter, and possessed twenty protoxylem-groups, the outer elements only of the xylem-rays being lignified. In this case the connexion between the protoxylem-groups of the mother-root, and of its branches, could not be determined, owing to the non-lignification of the protoxylem in the secondary roots, but in the second case of dichotomy observed the relation was satisfactorily worked out.

The dichotomous root in question was a side-branch of an earth-root, also from the Cambridge Botanic Garden. The main root was .65 cm. in diameter, possessed thirteen groups of protoxylem, and had lignified tracheides half-way to the centre. This produced a side-root, .25 cm. in diameter, possessing seven protoxylem groups, which shortly after its exit from the cortex of the mother-root underwent dichotomy. The products of the division were roots, .2 cm. in diameter; each possessed five groups of protoxylem, and its xylem-elements were lignified half-way to the centre. Fig. 30 shows a transverse section of the side-branch just after its exit from the main root; the xylem groups A, B, C are larger than the others. Fig. 29 shows the stele of the side-root undergoing dichotomy; the xylem groups A, B, C have each divided into two; A has given rise to 1 and 2, B to 3 and 4, and C to 5 and 6. Two new steles have been formed, each containing five groups of protoxylem.

This method of branching is certainly not general in the



roots of *Angiopteris*, but in connexion with these cases it may be of interest to mention that Mr. Seward has noticed apparent dichotomy in a petiole of an *Angiopteris* plant in the Botanic Garden at Leipzig.

The origin of the roots from the vascular strands of the stem has not been worked out, but it may be suggested that there is some relation between the roots and the central protoxylem in the stem stele. Casual sections showing the origin of the roots confirm the statement given by Mettenius of the presence of short, irregularly-shaped scalariform tracheides at the junction of the root and the stem-stele<sup>1</sup>.

The apices of the roots found on the plant under investigation were either absent, or in a too much decayed condition to afford any information as to their structure. My investigations into the root-apices obtained from the plant in the Cambridge Botanic Garden are confirmatory, as far as they go, of the views expressed in Koch's paper.

In the stouter air-roots a gap occupies the general position of the apical cells, and growth is carried on by the tissue of meristematic cells which surround the cavity. The gap, which originates near the apex by the disorganization of the large apical cells, persists for a considerable distance in the centre of the root-stele.

In somewhat smaller roots, several large cells are present at the junction of the root-cap tissue with that of the root proper, but it was not possible to determine their actual relation to each other. In the small branch-roots growth is apparently carried on by means of one apical cell, as described by Holle; this method of growth is indicated by the arrangement of the cells in the apical region, as seen in transverse section, in roughly concentric circles, but its existence can hardly be said to be definitely proved.

### III. Leaf.

There is no intention in the present paper to give any detailed account of the leaves of *Angiopteris*, but a few points

<sup>1</sup> Mettenius ('64), p. 519.

of interest may be mentioned with reference to the structure of the vascular tissue in the leaf-bases.

The method of division of the initial leaf-strands into a network has been already described, and the superficial part of the network is shown in Figs. 32 and 33. Fig. 33 represents the superficial tissue from the inner flat side of the leaf-base; the strands enclosed within the space *a, b, c, d*, are those which occupy the connate part of the stipules, and those at *p, q, r, s*, are branches making their way inwards to the petiole proper; the other petiolar bundles arise from the inner surface of the superficial vascular tissue and are not therefore represented in the diagram.

Fig. 32 shows the superficial vascular strands from the outer convex surface of the leaf-base; these are not represented in perspective, but as spread out over a plane surface. The cut ends of the bundles represented as lying along the arc *ab* are those of branches destined for the petiole; it is obvious that the petiole is thus continuous with the convex side of the leaf-base. At *s* are shown the fine strands belonging to the stipules; these form a network with long drawn-out meshes, and are accompanied by numerous secretory passages. The secretion, probably tannin, is contained in sacs or cells arranged in longitudinal rows; these are seen particularly well in a median section of the root-tip.

In the leaf the structure of the stele is that of the ordinary stem type, with the exception of the absence of the arc of protophloem. As in the stem, the vascular elements first differentiated form an arc of sieve-tubes on the side of the stele furthest from the centre of the organ, but in the case of the leaf these are of considerable size, and later form part of a continuous ring.

Soon after the formation of the protophloem, the protoxylem appears along the opposite side of the stele, one group only being formed in the case of the smaller, semi-lunar-shaped steles.

The ultimate bundles in the stipules show no recognizable phloem, but consist of a few tracheides, surrounded by small-celled parenchyma; frequently one tracheid only is present in the stipular steles.

CONCLUSION.

Since the anatomical structure of the stem of *Angiopteris evecta* has been described in most detail by Mettenius, it is of interest to compare with his results those now obtained.

The arrangement of the vascular strands in a series of inverted funnel-shaped zones is the same in both stems, but the closing up of the strands into continuous rings in the upper part of the stem, in the way described by Mettenius, must certainly be attributed to the unhealthy life of the plant for some time before its abandonment to the purposes of scientific research. There was not the slightest trace of such an altered structure in the apical regions of the plant from Ceylon.

Further, my investigations show that the leaf-trace bundles arise from superficial vascular tissue alone, and that the steles of the second zone do not share in the formation of the foliar strands, which are indefinite in number and position. This was not the case in the stem described by Mettenius, for here two bundles from the second zone passed off into each leaf, and the foliar strands were constant in number and arrangement. The difference in character of the foliar bundles in the upper and under leaves in Mettenius' plant is, of course, to be correlated with the difference in the vascular system in the stem in the two cases.

The stem just described presented definite dorsiventrality, both in its external characters and internal structure. The roots were produced in great numbers on a certain part of the lower surface of the stem, and diminished in number towards the apex. This latter fact is also noted by Mettenius, who attributes it to the altered conditions in the nutrition of the plant<sup>1</sup>.

The wearing away of the base of the stem described in the preceding pages did not allow of any conclusion as to the original structure of the vascular system in this part;

<sup>1</sup> Mettenius ('64), p. 517.



the arrangement must be examined in the case of some plant that has not undergone decay.

With regard to the internal structure of the stem-stele, the chief point of interest is the anomalous position of the protophloem.

The centrifugal growth of the phloem is contrary to that described for most other Ferns, and it would be of interest to examine the development of the steles in the other members of the Marattiaceae to determine whether in them a similar method of development is repeated. The histological structure of the stem-steles in *Kaulfussia* and *Marattia* has been described by Kühn, but no mention is made of the protophloem; this, however, is so indistinct in the mature bundles that, unless its position had been first determined in the young steles, its presence might well have been overlooked.

The number and position of the protoxylem-groups has been worked out for a complete transverse section of the stem, and the mesarch and endarch structure of the steles has been further confirmed by the position of the spiral and reticulate tracheides as seen in longitudinal sections of the steles.

In the apical regions of the stem the presence of several initial cells was satisfactorily demonstrated, but the exact number of these and their method of division still remain to be determined.

Investigations into the structure of the roots on the plant examined show that these are of one kind and correspond in structure to the earth-roots described by Russow; the stem was apparently destitute of air-roots.

Owing to the impossibility of obtaining the prothalli of *Angiopteris*, no examination could be made into the structure of the seedling stems; such work is of course necessary to render complete the account of the stem-anatomy.

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## EXPLANATION OF PLATES XXVIII AND XXIX.

Illustrating Miss Shove's paper on *Angiopteris*.

## PLATE XXVIII.

*Angiopteris evecta*.

Fig. 1. Piece of stem showing bare base, roots (R), and lower leaves. L, lenticels; P, petioles; S, stipules.

Fig. 2. Diagrammatic transverse section of mature stem after removal of leaves. R, roots; P.P, protophloem; P.X, protoxylem.

Fig. 3. Longitudinal section of young frond. s, outer stipule; s<sup>1</sup>, inner stipule.

Fig. 4. Transverse section of young frond taken along the line AB in Fig. 3. P, circinnately coiled petiole; s<sup>1</sup>, inner stipule; s, outer stipule. Line A<sup>1</sup>B<sup>1</sup> corresponds in position to line AB.

Fig. 5. Portion of the vascular network from the upper side of the stem. R, roots.

a,	strands belonging to leaf VI.
b	" " " V.
c	" " " IV.
d	" " " II.
e	" " " I.
f	" " " III.

Dotted lines show strands of third zone. Shaded strands belong to second zone.

A, B, C, D, points of union of strand from second zone, compensating for the departure of the strands e of the first zone into leaf I, with the vascular tissue of first zone. M, N, P, similar points in case of strand compensating for the departure of the strands c into leaf IV.

E, F, G, H, points of union of strands of third zone with those of second zone.



Fig. 6. Portion of the superficial vascular tissue from the under side of stem R, roots.

Shaded strands are those belonging to second zone.

<i>a</i> ,	strands	belonging	to	leaf	II.
<i>b</i>	"	"	"	"	I.
<i>e</i>	"	"	"	"	III.
<i>c</i>	"	"	"	"	IV.
<i>d</i>	"	"	"	"	V.

Fig. 7. Vascular strands of leaf-base from upper side of stem. A, B, branches to two other leaves.

Fig. 8. Vascular strands of leaf-base from lower side of stem. R, root.

Figs. 9, 10, 11, 12. Series of transverse sections of leaf-base showing branching of steles. R, root. Series is that of leaf I in Fig. 6.

Figs. 13-18. Series of transverse sections from base of stem. Shaded steles are those derived from branching of original stele (s) in extreme base of stem.

Fig. 19. Transverse section of stelar tissue on side nearest the centre of stem. P.X, protoxylem; s, sieve-tubes; C, cortex.  $\times 100$ . Arrow points to centre of stem.

#### PLATE XXIX.

Fig. 20. Transverse section of stele in air-root. C, cortex; E, endodermis; P.X, protoxylem; P, phloem; R, resin-passage; A, unligified tracheides.  $\times 100$ .

Fig. 21. Transverse section of large stem stele. P.X, protoxylem; s, sieve-tubes.  $\times 25$ .

Fig. 22. Transverse section of earth-root. E, endodermis.  $\times 40$ .

Fig. 23. Transverse section of young stem stele. s, sieve-tubes; P.P, protophloem; P.X, protoxylem.  $\times 100$ .

Fig. 24. Transverse section of air-root. X, peripheral group of ligified tracheides; A, unligified tracheides; P, phloem; E, endodermis.  $\times 40$ .

Fig. 25. Transverse section of young stem stele. P.X, protoxylem; P.P, protophloem; s, sieve-tubes.  $\times 40$ .

Fig. 26. Transverse section of small circular stem stele. P.X, protoxylem; s, sieve-tubes.  $\times 40$ .

Fig. 27. Diagrammatic transverse section of lateral roots. X, xylem rays in mother-root; P, pith; E, endodermis; C, cortex in lateral roots.

Fig. 28. Transverse section of stelar tissue on outer side. s, sieve-tubes; P.P, protophloem; X, xylem.  $\times 100$ . Arrow points to centre of stem.

Figs. 29, 30. Diagrammatic transverse sections of dichotomising root. A, B, C, larger rays of xylem in main root:

1, 2,	products	of	division	of	A.
3, 4,	"	"	"	"	B.
5, 6,	"	"	"	"	C.

Fig. 31. Protoxylem elements from stem stele. s, spiral tracheid; R, reticulate; K, scalariform.

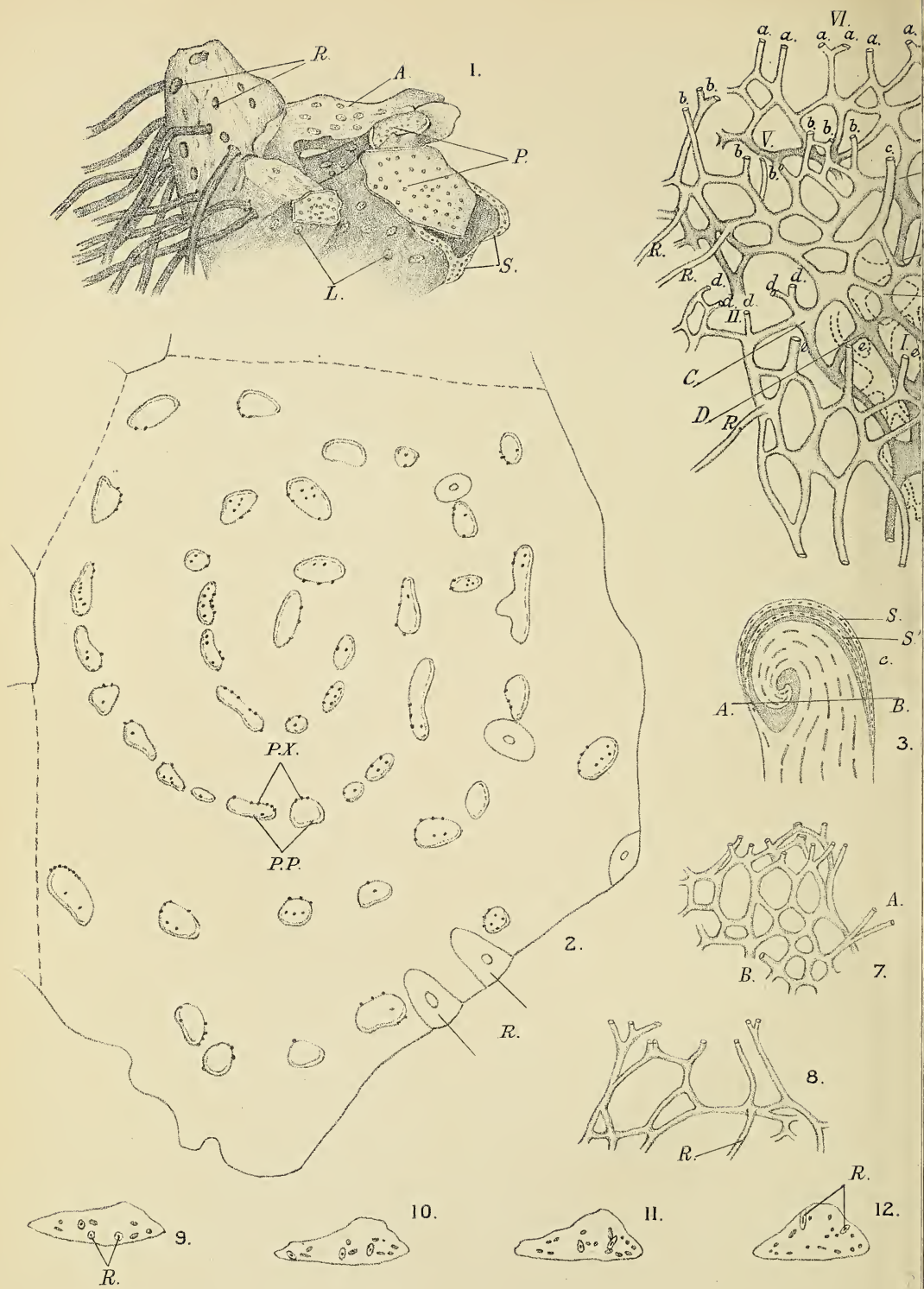
Fig. 32. Superficial vascular network from convex side of leaf-base. *a-b*, petiolar bundles; s, stipular bundles.

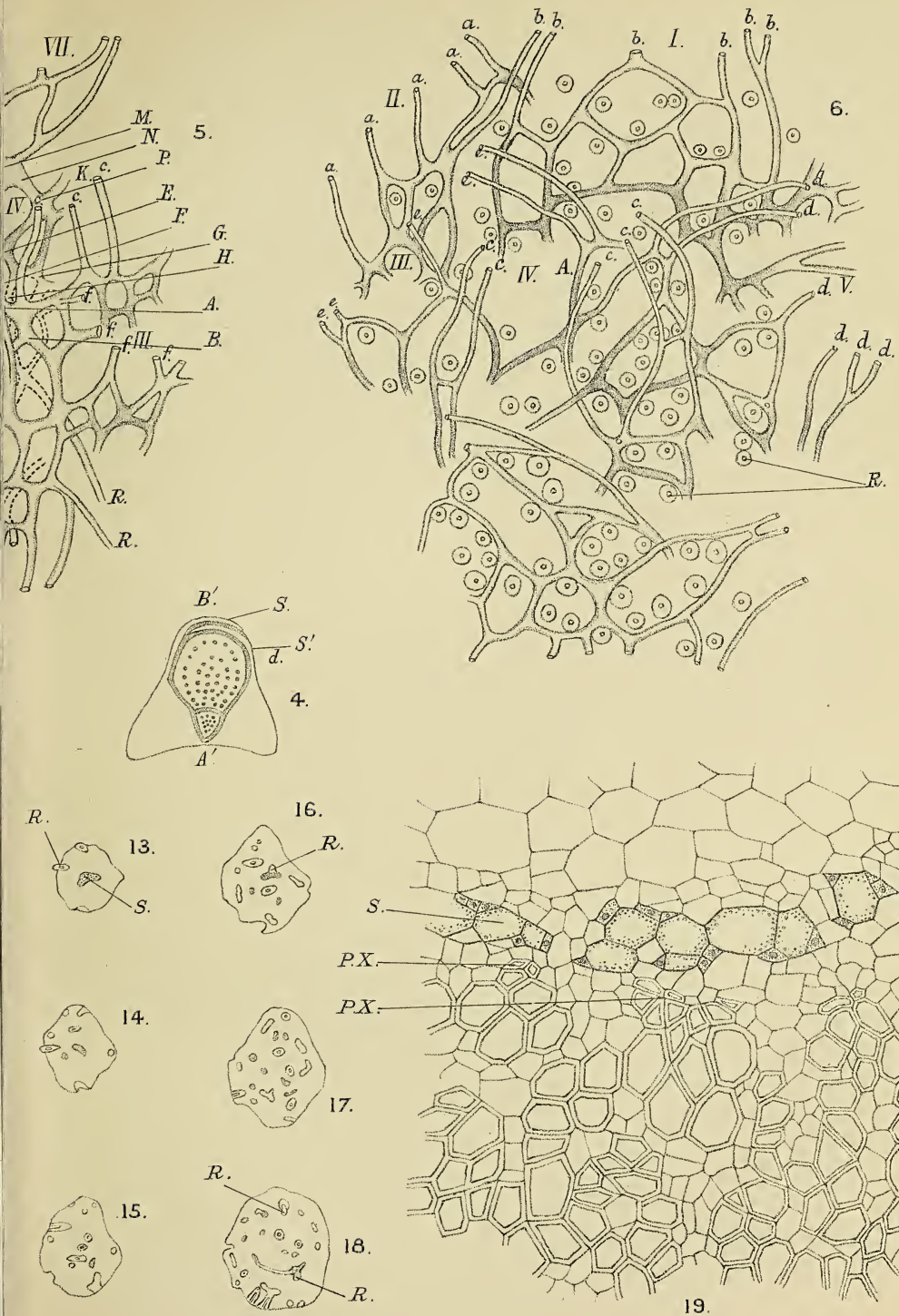
Fig. 33. Superficial vascular strands from flat side of leaf-base. *a, b, c, d*, encloses strands which occupy partition joining stipules; *p, q, r, s*, strands going to petiole.





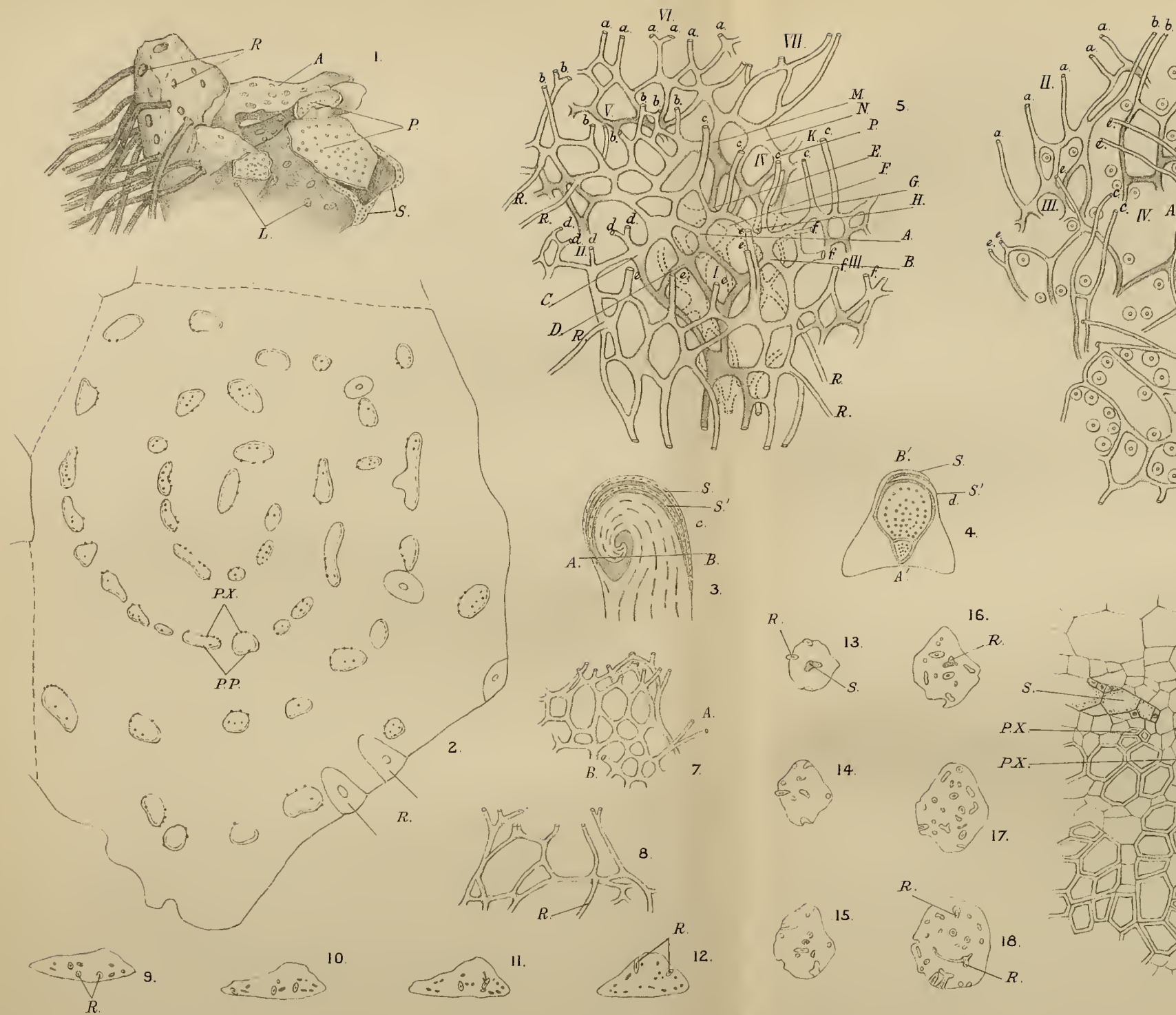












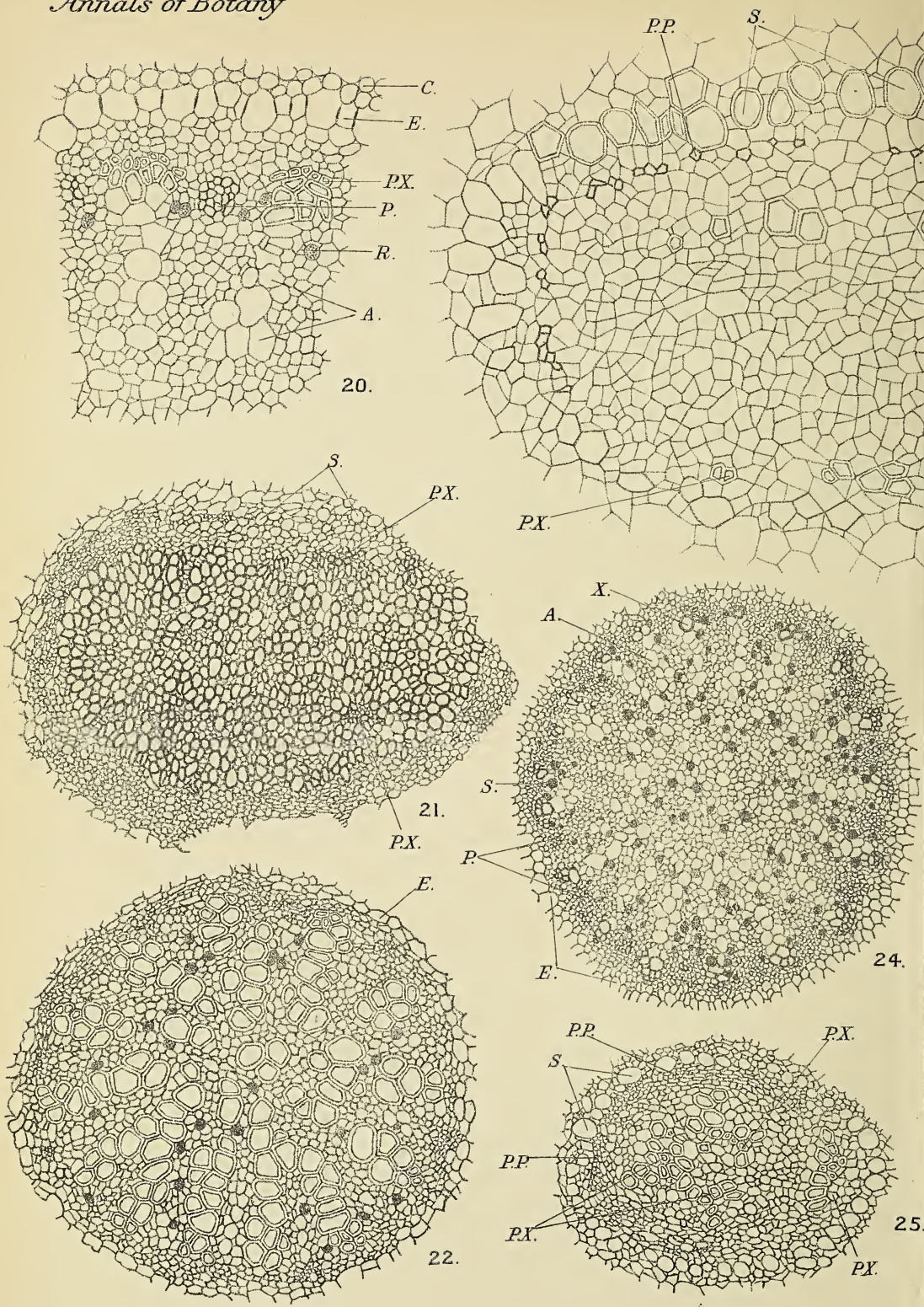
RF Shove, del.

SHOVE.—ANGIOPTERIS.



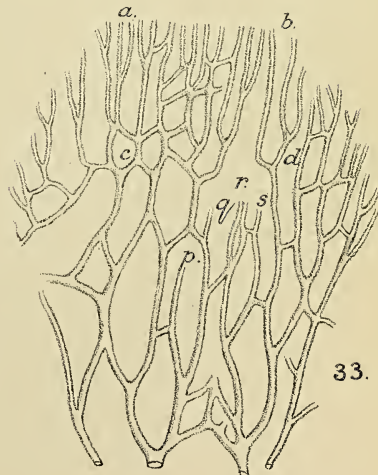
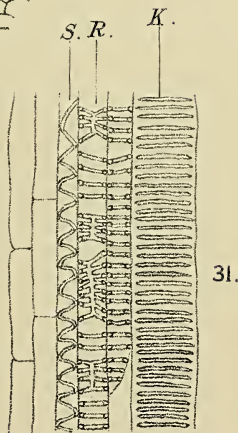
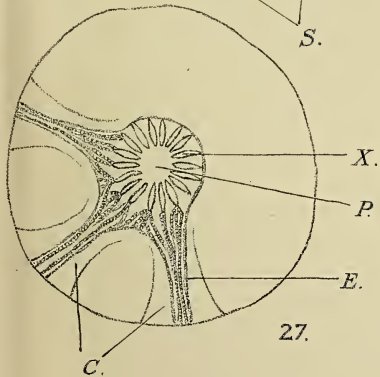
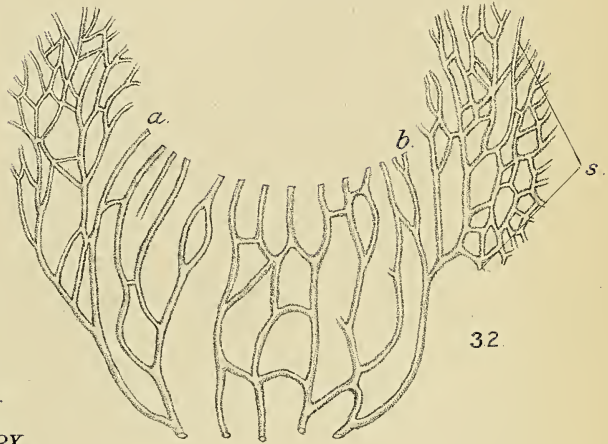
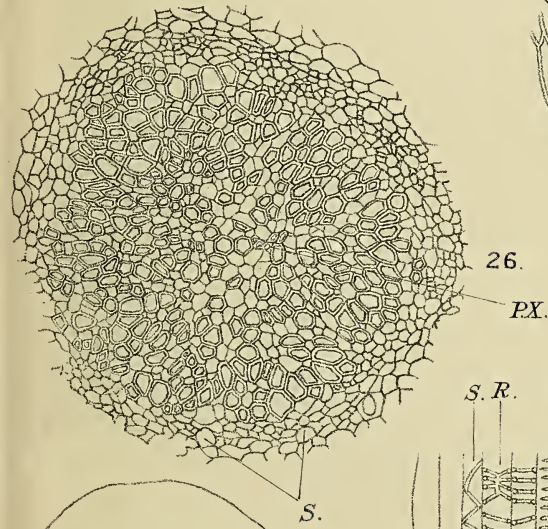
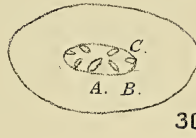
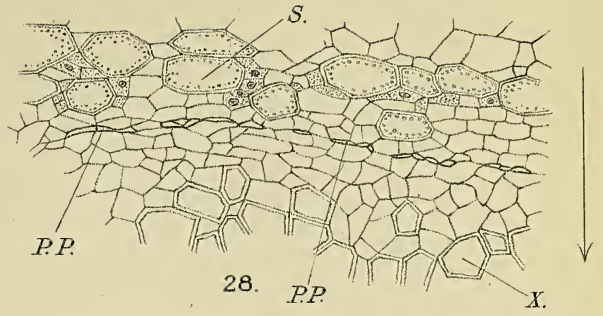






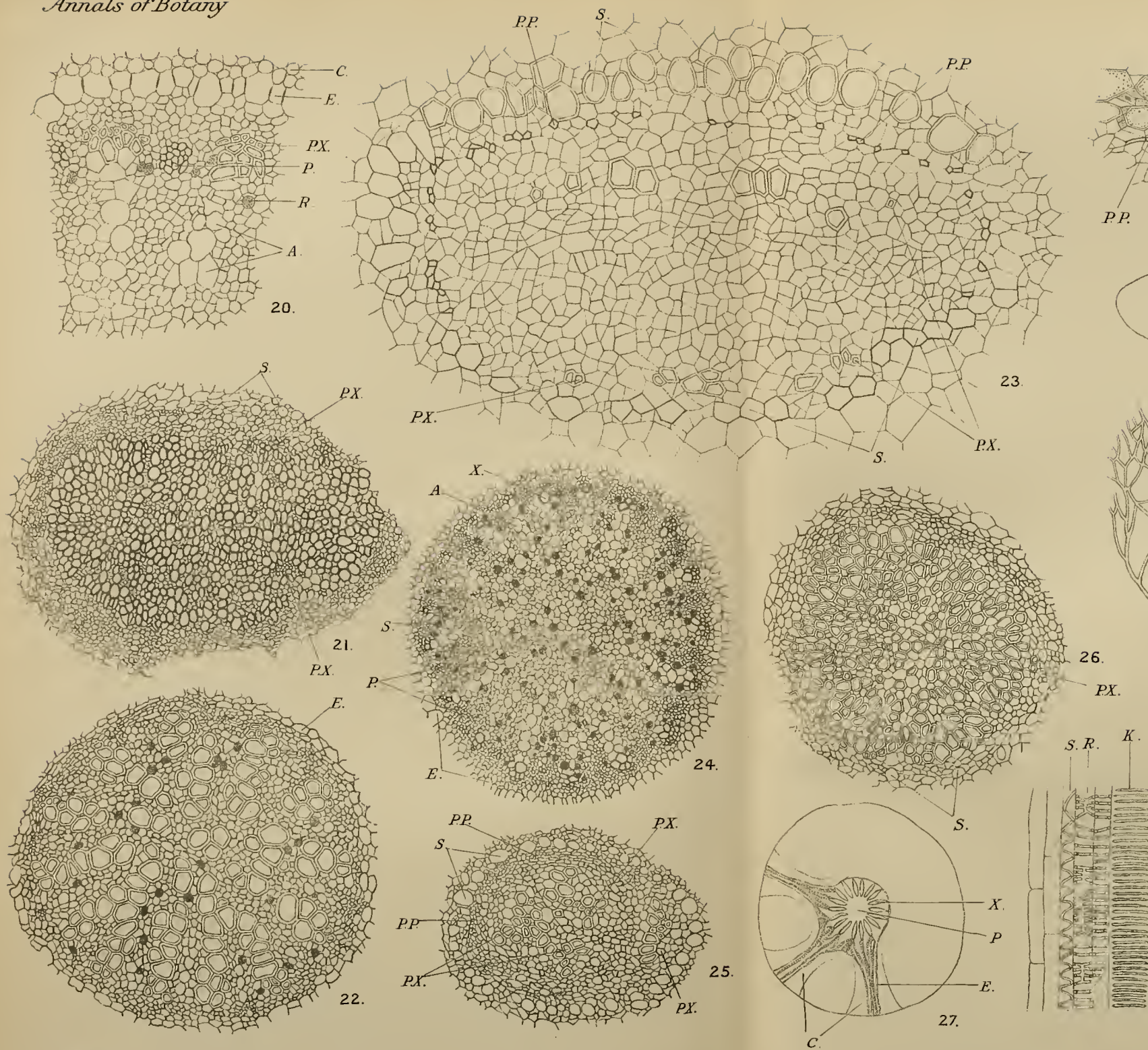
R.F. Shove, del.  
W. Tams, phot.











R.F. Shove, del.  
W. Tams, phot.

SHOVE — ANGIOPTERIS.



# Double Fertilization in a Dicotyledon— *Caltha palustris* <sup>1</sup>.

BY

ETHEL N. THOMAS.

—♦—  
With Plate XXX.  
—♦—

THIS striking and hitherto unknown phenomenon of double fertilization was, I think, first discussed in England after the publication of Professor Léon Guignard's paper on *Lilium Martagon* and *L. Pyrenaicum* in the spring of 1899 <sup>2</sup>. In August of the preceding year, however, Professor S. Nawaschin had given an account of his new researches on *L. Martagon* and *Fritillaria tenella* before the Russian Scientific Congress held at Kief <sup>3</sup>.

It will be remembered that upon the expulsion of the contents of the pollen-tube into the embryo-sac of any typical Angiosperm, one of the two generative nuclei fuses with the nucleus of the oosphere, but the other has never been satisfactorily accounted for, and it was presumed that it became disorganized as the synergids do. Nawaschin, however, found in the plants mentioned that this second generative nucleus

<sup>1</sup> E. N. Thomas, 'On the presence of vermiform nuclei in a Dicotyledon.' Note, Annals of Bot., June, 1900.

<sup>2</sup> M. L. Guignard, 'Sur les anthérozoïdes et la double copulation sexuelle chez les végétaux angiospermes.' Comptes rendus, t. cxxviii, Avril, 1899.

<sup>3</sup> S. Nawaschin, 'Resultate einer Revision der Befruchtungsvorgänge bei *Lilium Martagon* und *Fritillaria tenella*.' Bull. de l'Acad. des Sc. de St. Pétersbourg, 1898, novembre.



travels to the middle of the sac, and there coalesces with the upper polar nucleus, and then both fuse with the lower polar nucleus. Guignard agrees with this, and furthermore states that the polar nuclei may have approached<sup>1</sup> one another before the generative nucleus joins them, and gives many figures showing the triple fusion. He gives instances also in which, when the polar nuclei are still separate, the generative nucleus joins the lower one. However this may be, the definitive nucleus which later gives rise to the endosperm is the product of the fusion of three nuclei of very different origin. The upper polar nucleus is sister-nucleus to that of the oosphere, and were it not for the presence of the lower polar nucleus, which is vegetative in character, the fusion of the second generative nucleus with the upper polar nucleus would be a true fertilization precisely equivalent to the fertilization of the oosphere by the male nucleus.

In addition to this most important discovery, both observers found that the generative nuclei had a very remarkable shape. They are long and narrow, and twisted in every conceivable manner, so that the term vermiform has been applied to them, and their appearance suggested to Guignard their strict analogy to antherozoids. The contortions are particularly noticeable in the second generative nucleus on its journey to the polar nuclei, and no doubt indicate the wriggling movement by which it travels through the sac.

When these accounts were published, Miss Sargent found that she had preparations of *Lilium Martagon* made years before which showed the vermiform nuclei and double fertilization extremely well<sup>2</sup>, and as she still possessed the material which furnished these slides, she proposed that I should cut some of it to see how readily this stage could be obtained. As it was not convenient in some ways to work at Reigate, Professor Farmer very kindly consented to my working at the Royal College of Science, South Kensington, and throughout has given me the greatest assistance. I went there in May of

<sup>1</sup> Comptes rendus, p. 4 of separate copy.

<sup>2</sup> Sargent, Proc. Roy. Soc., May, 1899.

last year, quite prepared to extend my researches to other plants if *L. Martagon* proved fruitful speedily. The material must have been extremely rich in this stage, and very well fixed, for both hand sections and microtome series yielded very good instances at once.

As the matter seemed fairly well established for typical Monocotyledons, it became desirable to see if the second fertilization took place in Dicotyledons also. At Professor Farmer's suggestion I sectioned *Caltha palustris* among other plants, and this seemed to be a favourable object for study, although too old. I therefore went out in search of fresh material, and obtained it from Hackbridge, where it grew very abundantly. The ovaries were pickled on one or two bright mornings (11.30 and 12.30) at the end of May, some in absolute alcohol and 25% acetic acid, and some in strong Flemming's fixative. Those chosen were mostly from fresh flowers, but of different ages, as far as one could judge from their appearance. I know now, since carefully marking flowers of *Caltha* this spring, that they remain open for at least seven days, and this material must therefore have included ovaries of very different ages. Microtome sections were cut, for the most part, from the Flemming material, and hand sections from that fixed in absolute alc. and 25% acetic acid, but curiously enough the most interesting preparations were obtained from microtome sections of ovaries from the latter material, so that these are not so well preserved as many of the less important stages. Hand sections were stained in Methyl Green and Fuchsin, but the microtome sections were stained with Flemming's Triple Stain or Ehrlich's Haematoxylin.

The relatively large size of the oval-shaped embryo-sac and small size of the nuclei renders it difficult, and perhaps almost impossible, to obtain preparations in which the protoplasm is not somewhat strained. In some of the younger stages, in which the polar nuclei are only just in contact with one another, the sac is much smaller, and well filled with protoplasm; and in these the preservation is quite good. Later, the embryo-sac enlarges considerably, and the vacuoles

get so big that only a few slender strands of protoplasm bridge the central cavity.

The polar nuclei have generally completely fused before the entrance of the pollen-tube, but there is evidently a considerable amount of latitude in point of time, for I have found polar fusion in embryo-sacs of very different ages. The greater number of cases have been seen in sacs of the same age as that drawn in Fig. 1, or slightly older. Here the ovule is evidently quite young, for the integuments have not yet closed over the nucellus, while within the embryo-sac all the nuclei, even those of the antipodals, are in good condition, and all possess, as a rule, one central nucleolus. The antipodals are large pear-shaped cells and, at this period, very often contain two nuclei apiece produced by karyokinesis, but later, when the centre of the sac is occupied by one large definitive nucleus, the nuclei of each antipodal cell have run together to form a large irregular mass, containing many nucleoli. Frequently, however, the ovule is much older than this at the time of polar fusion, and in one instance the pollen-tube was seen in the micropyle at that period. This did not mean the early arrival of the pollen-tube, for the contents of the embryo-sac showed signs of age. Its cavity was immensely larger than that of the sac from which Fig. 1 was drawn, and its oosphere had increased in size while the synergids were somewhat degenerated; the antipodals also were bigger, and their centre occupied by a large deeply-stained nuclear mass. The coalescence of the polar nuclei is evidently taking place very late, for endosperm and embryos show most commonly in the other embryo-sacs of this ovary.

When the generative nuclei are extruded from the pollen-tube<sup>1</sup> they are very minute and stain heavily. Very often they are merely oblong, or lens-shaped, but sometimes they

<sup>1</sup> The swollen end of the pollen-tube is provided with a sharp point, evidently for boring through the nucellar tissue. I have seen the pollen-tube turn at right angles when passing through the nucellar tissue so that it entered the embryo-sac laterally.



are like small dumb-bells or may even have the form of a somewhat straight S. Their chromatic elements are so densely packed at this time that no structure can be made out. Although not able to follow it out with any certainty, I have always been strongly under the impression that it was the first nucleus that left the pollen-tube which fertilized the polar nuclei, for the polar fertilization is always much more advanced than that of the oosphere. I have read since that Professor Guignard finds this to be so in the Monocotyledons that he studied, and therefore it is extremely likely that it is the case in *Caltha* also. This may partly account for the fact that the process has been overlooked for so long. For when fertilization of the oosphere takes place, the polar fertilization may be too far advanced to be noticeable by any one not on the lookout for it.

By the time the vermiform generative nucleus has reached the middle of the sac, it has enlarged from an extremely small body to a nucleus of very considerable size, and its chromatic elements now show particularly clearly as small widely-scattered granules. The nucleus which fertilizes the oosphere, on the contrary, increases very little in size, and its chromatin still remains somewhat tightly packed, so that it is always very dark and shows merely a coarsely granular structure.

The result of the two fusions taking place at a slightly different time is of course that one rarely gets very good instances of both in the same sac. Where the ovum and male nucleus are actually coalescing, as shown in Fig. 2, the polar fertilization is in a late stage, so that there is only one end of the vermiform nucleus clearly distinguishable from the general polar mass (Figs. 3 and 4). In the sac from which Figs. 8 and 9 are taken, the male nucleus has only just reached the oosphere, and therefore, as one would expect, the vermiform nucleus, although tightly wound round the definitive nucleus, and indeed coalescing with it in parts, yet shows several of its sharp turns. In Figs. 6 and 7, however, we have a much better instance of the vermiform nucleus, for here it is only lightly applied to the definitive nucleus, and fusion has

not begun, so that we can follow its shape from end to end. It makes one large coil almost back upon itself, by means of which it rises on to the surface of the definitive nucleus.

I have seen a great many other sacs, in which it was perfectly evident that the second fertilization had taken place, but fusion had gone too far for one to be able to distinguish the vermiform nucleus with precision.

It will be noticed in the illustrations that I have given that the definitive nucleus does not present a rounded, even contour, but that it often appears as if the polar nuclei of which it is composed still retain their individuality to some extent. This is most marked in the fusion shown in Fig. 4, where the definitive nucleus still contains several nucleoli, and at one side the polar nuclei are quite separate. The definitive nuclei shown in Figs. 7 and 9 contain only one nucleolus, but there are deep indentations in their outlines which probably mark the boundaries of their component nuclei. This seems at first a little difficult to reconcile with the very numerous instances I have seen of one large, almost perfectly round, definitive nucleus, in sacs not yet fertilized. It is quite possible that some at any rate of these have passed the stage at which fertilization is possible, and that they then fully complete their earlier fusion. Some, however, certainly do unite completely, before fertilization, for I have seen a vermiform nucleus fusing with a single large definitive nucleus containing one central nucleolus.

The synergids also differ somewhat in their condition at the time of the entrance of the pollen-tube. Generally they seem fairly round and healthy, but very soon become disorganized. Sometimes they possess a somewhat broken outline, even when the pollen-tube first enters.

The antipodals, on the contrary, remain large and important-looking after fertilization has taken place, as Dr. Westermaier<sup>1</sup> has found to be the case in many members of the order Ranunculaceae.

<sup>1</sup> Dr. M. Westermaier, *Zur Embryologie der Phanerogamen, insbesondere über die sogenannten Antipoden.* Halle (Acad. Leop.), 1890.

The definitive nucleus divides by mitosis, and often forms a considerable amount of endosperm before the oosphere divides. The first wall formed in the latter is at right angles to the long axis of the sac.

All the practical work in connexion with this paper was done at the Royal College of Science last summer, but although I was convinced that a double fertilization, by means of vermiform nuclei from the pollen-tube, took place, I was unwilling to publish at the time, as I had not found a perfect and unmistakable fertilization of the oosphere by the male nucleus, in the same sac with the fertilization of the definitive nucleus. In examining my slides again this spring, however, I came across a perfectly satisfactory fusion of the oosphere and male nucleus previously overlooked (see Figs. 2 and 5). In the same sac is a sufficiently good polar fertilization, although only a small portion of the vermiform nucleus is to be seen, but, as I explained before, it seems unlikely that an equally felicitous stage of both will be found in the same sac.

My thanks are due to Miss Sargent for very kindly giving me her advice on some slides that I submitted to her.

Since writing this paper, I have seen Professor Nawaschin's recently issued account of double fertilization in Dicotyledons. As a representative of the Ranunculaceae he has examined *Delphinium*, and although not able to find the male generative nuclei free in the embryo-sac, he saw them as vermiform structures in the pollen-tube, and again when fusing with the nucleus of the oosphere and definitive nucleus respectively. In two respects *Delphinium* resembles *Caltha*: its polar nuclei unite before fertilization, and the fertilized ovum remains undivided for a considerable time. There are no figures of this plant.

Professor Nawaschin's paper is, however, more particularly concerned with two Composites, *Helianthus annuus* and *Rudbeckia speciosa*. In both these plants also, the polar nuclei coalesce before fertilization. In *Rudbeckia*, as I found in *Caltha*, the fusion of the one male generative nucleus with the



definitive nucleus *takes place before that of the other with the oosphere* (*l.c.*, Fig. 1, B).

The spermatozoids of *Rudbeckia*, and also of *Helianthus*, show the characteristic shape and granular structure extremely well. In *Helianthus* they are very long and twisted like a corkscrew, while they are shorter and thicker in *Rudbeckia*, but show their structure even better.

Nawaschin differs from Guignard in not having seen the spermatozoids of *Lilium* either homogeneous or with a spiral ribbon at any period. In *Caltha* they are so extremely small, when they first come from the pollen-tube, and take the stain so heavily, that I was not able to make out any structure whatever. They appeared homogeneous, but this may have been due to difficulties in observation. Later, when the one spermatozoid has reached the definitive nucleus, it is immensely swollen, and has a particularly light, spongy structure with minute scattered granules.

Professor Nawaschin has also made discoveries of the utmost importance in Orchids. These plants form no endosperm, and it is therefore of great theoretical significance to find that, even when the embryo has attained a considerable size, the three nuclei associated together in the centre of the sac have not coalesced. Two of these are the unfused polar nuclei, and there can be little doubt that the third is a spermatozoid, although it has lost its characteristic form (*l.c.*, Fig. 2).

Professor Nawaschin has always believed that the fusion of the second male generative nucleus with the definitive nucleus is a true fertilization, and he takes these results as in a great measure proving it. For our knowledge of the nature of true fertilization is so limited that at present we can say little more than that it is followed by division. And in this respect the result of the two fusions is identical.

I have also read Professor Guignard's paper on Tulips, in which he mentions that he has seen the double fertilization in some Dicotyledons, including Ranunculaceae.

## EXPLANATION OF FIGURES IN PLATE XXX.

Illustrating Miss Thomas's paper on the Fertilization of *Caltha*.

Abbreviations: *n. of o.*, nucleus of the oosphere; *s.*, synergid; *g. n<sup>1</sup>.*, first generative nucleus, that which fertilizes the oosphere; *g. n<sup>2</sup>.*, second generative nucleus, that which fertilizes the definitive nucleus; *d. n.*, definitive nucleus; *ant.*, antipodal cell; *p<sup>1</sup>* and *p<sup>2</sup>*, polar nuclei.

Fig. 1. Young embryo-sac with polar nuclei in the first stage of fusion (*p<sup>1</sup>* and *p<sup>2</sup>*). × 450.

Fig. 2. Embryo-sac at the fertilization period. Nucleus of oosphere and first male generative nucleus, coalescing. The second generative nucleus is in a more advanced stage of fusion with the definitive nucleus. Antipodal cell displaced. × 245.

Fig. 3. Definitive nucleus with second generative nucleus from Fig. 2, under greater magnification. This section includes but a thin slice of these nuclei. They show more plainly in the next section. × 550.

Fig. 4. Next section of the definitive nucleus shown in Fig. 3. The polar nuclei of which it is composed are evidently not completely fused. It contains at least three nucleoli. The somewhat torn nucleolus to the extreme left seems to be a section of one of the others which has drifted there. It, however, prevents one from following out the vermiform generative nucleus which shows just below it. × 550.

Fig. 5. Upper part of embryo-sac shown in Fig. 2 under greater magnification. × 550.

Fig. 6. Portion of embryo-sac with much coiled second male generative nucleus fertilizing the definitive nucleus. The latter contains only one nucleolus, but has an irregular outline with one sharp constriction which may mark the point of fusion of its polar nuclei. × 200.

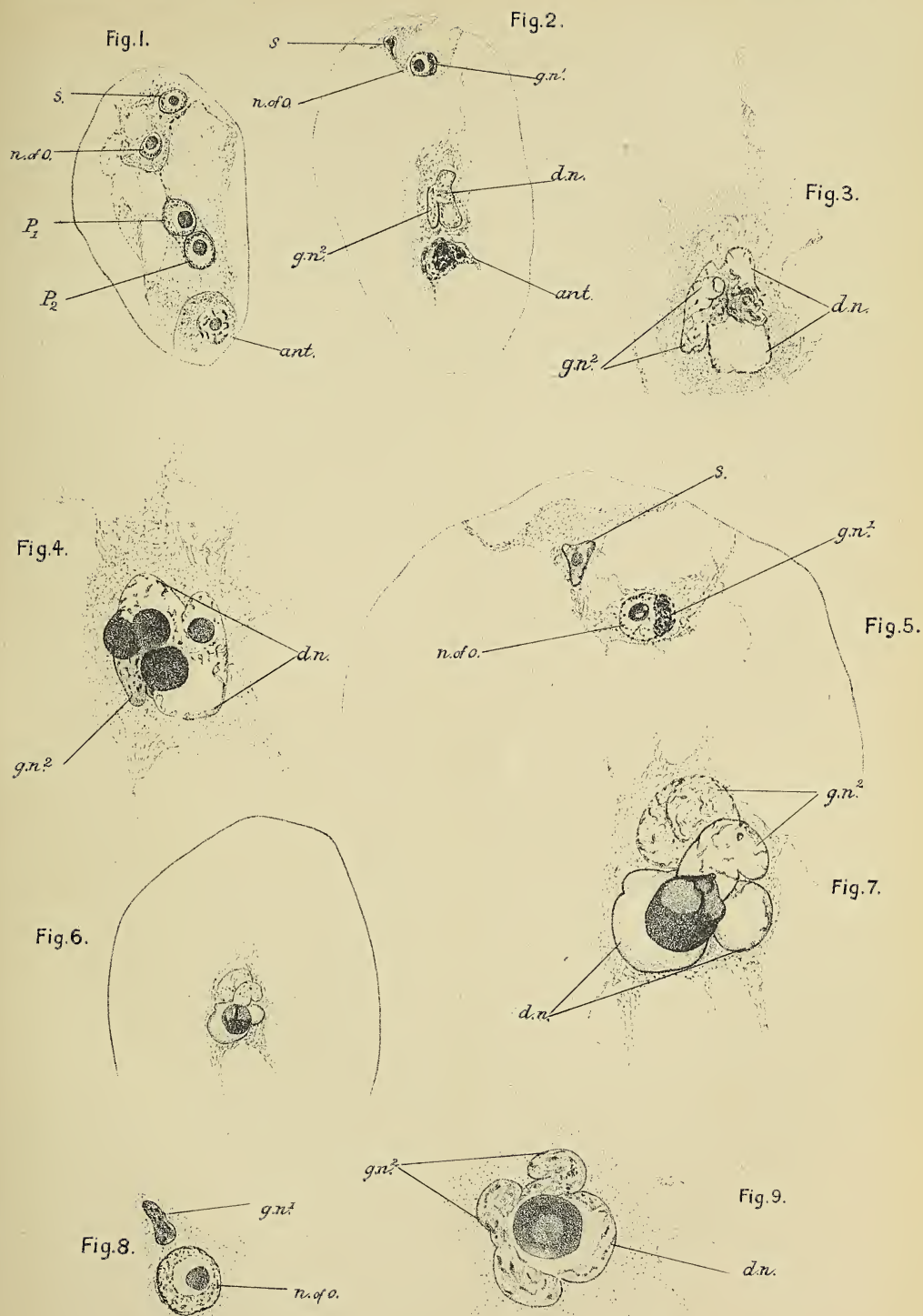
Fig. 7. Definitive group from Fig. 6, under greater magnification. × 550.

Fig. 8. Oosphere with male generative nucleus just touching its nucleus. × 550.

Fig. 9. Definitive group from same sac as furnished Fig. 8. The second generative nucleus is in an advanced stage of fusion with the definitive nucleus, but still shows its vermiform shape very distinctly. × 550.









## NOTES.

STATIC DIFFUSION OF GASES AND LIQUIDS IN RELATION TO THE ASSIMILATION OF CARBON AND TRANSLOCATION IN PLANTS<sup>1</sup>. By HORACE T. BROWN, F.R.S., LL.D., and F. ESCOMBE, B.Sc., F.L.S.—This paper is intended to be the first of a series descriptive of the work carried out by the authors in the Jodrell laboratory on the fixation of carbon by green plants, and deals mainly with the purely physical processes by which atmospheric carbon dioxide gains access to the active centres of assimilation.

The new evidence which F. F. Blackman brought forward in 1895 in favour of the gaseous exchanges of leaves taking place exclusively through the stomatic openings, presents at first sight certain difficulties of a physical nature, which have led to an examination of the whole question of the free diffusion of carbon dioxide at very low tension, and under a set of conditions very different from those under which the previous determinations of the coefficient of diffusion of carbon dioxide and air have been made by Loschmidt and others, where the gases were initially of equal tension, and the ratios of mixture departed widely from those of ordinary atmospheric air. The inquiry has led to the discovery of some new facts connected with the static diffusion of gases and liquids, which are of considerable interest, not only from the physical point of view, but from the explanations they suggest of certain natural processes which are primarily dependent on diffusivity.

The method employed in the first instance for the determination of the diffusivity of atmospheric carbon dioxide was one of *static diffusion* down a column of air of a definite length, towards an absorptive surface at the bottom of the column. When a static con-

<sup>1</sup> Abstract, from the Proceedings of the Royal Society, Vol. lxi.



dition has been established, there is a steady flux of the carbon dioxide down the air column which may be quantitatively investigated by the same simple mathematical treatment as the 'flow' of heat in a bar when the permanent state has been reached, or the 'flow' of electricity between any two regions of a conductor maintained at a constant difference of potential.

By a long series of experiments of this nature it was found that the diffusivity constant,  $k$ , for very dilute  $\text{CO}_2$  does not materially depart from the value assigned to it by Loschmidt and others, when experimenting with much higher ratios of mixture, and that the difference is certainly not of sufficient magnitude to be taken into serious account in the study of the natural processes of gaseous exchange in the assimilating organs of plants.

In the static diffusion of a gas, vapour, or solute, as the case may be, the amount of substance diffusing in a given time, all other conditions being the same, is directly proportional to the sectional area of the column. It is found, however, that if the flow is partially obstructed by interposing at any point in the line of flow a thin septum pierced with a circular aperture, the rate of flow across unit area of the aperture is greater than it would be across an equal area of the unobstructed cross-section of the column at this point. If the margin around the aperture has a width of at least three or four times its diameter, the rate of flow is now found to be directly proportional to the *linear dimensions* of the aperture and not to its area, so that the velocity of flow through unit area varies inversely as the diameter.

A large number of experiments on the diffusion of carbon dioxide, water-vapour, and sodium chloride in solution, are given in support of this proposition. All these show that the rate of diffusion across such a septum, all other conditions being the same, is directly proportional to the diameter of the aperture, and not, as might have been expected, to its area.

Exactly the same result is obtained when small circular disks of an absorbent, such as a solution of caustic alkali, are surrounded by a wide rim and exposed to *perfectly still* air, the amount of carbon dioxide absorbed under these conditions being proportional to the *diameters* of the disks.

If, however, there are any sensible air currents the absorption becomes proportional to the areas.

These two sets of phenomena may be explained as follows :—

In the case of the absorbing disk in perfectly still air, the convergent streams of carbon dioxide creep through the air towards the absorbing disk, establishing a steady gradient of density, and this creep will be a flux perpendicular to the lines of equal density, which form curved surfaces or 'shells' surrounding the disk and terminating in the rim. The state of things is exactly analogous to the electric field in the neighbourhood of a conductor of the same shape and dimensions as the absorbent disk<sup>1</sup>. In the case of the gas, the curves or 'shells' of equal density are the analogues of the similarly curved surfaces of equipotential above the electrified disk, whilst the converging lines of creep or flux of the gas are the analogues of the lines or tubes of force which bend round into the disk as they approach it.

If we consider two such absorbent disks of different diameters, the curved surfaces in each system corresponding to a given density will be found at actual distances from the disks which are in the same proportion to each other as are the diameters of the disks. In other words, the gradient of density on which the rate of flow depends will be proportional to the diameters of the disks, which is exactly what is found experimentally.

This case of an absorbent disk is the exact converse of one which has been theoretically investigated by Stefan, viz. the conditions of evaporation of a liquid from a circular surface. He found that the lines of flux of the vapour proceeding from the surface of the liquid must be hyperbolas, whilst the curved surfaces of equal pressure of the vapour must form an orthogonal system of ellipsoids, having their foci, like the hyperbolas, in the bounding edges of the disk. This was a purely mathematical deduction which has never been verified experimentally, but it will be seen that the exactly converse phenomena of diffusion are in complete agreement with it.

In the other case of a diffusive flow through a circular aperture in a diaphragm, the lines of flow, which are *convergent* as they approach the aperture, bend round their foci situated in the edges of the disk and form a *divergent* system on the other side. If the chamber into which they pass is a perfectly absorbent one, and is sufficiently large, there will be formed on the inner side of the

<sup>1</sup> The authors are indebted to Dr. Larmor for this suggestion of the electrostatic analogy.

diaphragm a system of density shells similar to those outside, but with the gradient of density centrifugally instead of centripetally arranged. This system of shells is termed negative, and is as effective as the outer positive system in regulating the flow according to the 'diameter law,' so that this law will still hold good even if the outer air currents are sufficient to sweep away the external positive shells altogether.

All the known facts of diffusion through circular apertures in a diaphragm are in complete accord with the above explanation, which is fully elaborated in the original paper.

By diffusing colouring matter through apertures in a septum, under such conditions as to prevent convection currents, the 'density shells' have been rendered visible, and it has been shown that their ellipsoidal form is exactly that which is demanded by the above hypothesis. Moreover, this method gives an experimental demonstration of the more rapid projection of the diffusing particles from the edges of the aperture than from a point nearer its centre, a fact completely in harmony with the deduction of Stefan regarding the evaporation of liquids under analogous conditions.

The various cases which present themselves in practice with regard to the rate of diffusion through single apertures in a diaphragm are then discussed from the above point of view, and simple formulae for the determination of this rate for single and double systems of density shells are established: (1) for cases where the thickness of the diaphragm is negligible, and (2) for other cases where the apertures become more or less tubular. In a subsequent section of the paper it is shown how closely the observed facts conform to these deductions, and that in static diffusion through apertures in a septum we have a new and accurate method for the determination of the diffusivity constants of atmospheric  $\text{CO}_2$ , of the vapours of liquids, and of substances in a state of solution.

Since the velocity of the diffusive flow through unit area of an aperture in a diaphragm varies inversely with the diameter, it might reasonably be expected that a diaphragm could be so perforated with a series of very small holes arranged at suitable distances from each other, as to exercise little or no sensible obstruction when it was interposed in a line of diffusive flow, although the aggregate area of the small holes might represent only a small fraction of the total area of the septum. Multiperforate diaphragms of this kind were



found to possess all the remarkable properties which had been anticipated.

The material used for the septa was very thin celluloid, which was perforated at regular intervals with holes of about 0.38 mm. in diameter. Details of a number of experiments with such diaphragms are given, in which it is shown that they may be so arranged as to produce but little obstructive influence on the diffusive flow of a gas when the total area of the apertures amounts only to about 10 per cent. of the area of the septum, and that nearly 40 per cent. of the full diffusive flow may be maintained when the number of the apertures is so far reduced as to represent an area of only 1.25 per cent. of the full area of the septum.

The explanation is to be found in the local intensification of the gradient of density in the immediate neighbourhood of the diaphragm, and which does not extend to the column away from the apertures. This disturbance of gradient is brought about by the rapid convergence of the lines of flux, and their divergence on the other side, with the consequent formation of a system of 'density shells' over each aperture. A system of perforations of this kind may be compared with a system of conductors electrified to a common potential, the density of the diffusing substance above the apertures corresponding to electric potential, and the non-absorbing portions of the diaphragm to a surface formed by lines of electric force. Just as the electric capacity of a plate is not much reduced by cutting most of it away, so also is it possible to block out a large portion of the cross-section of the diffusing column without materially altering the general static conditions on which the flow depends.

The importance of these results in relation to diffusion through porous septa is next considered, diffusion through a thin porous septum being only an extreme case of free diffusion through a multi-perforate diaphragm, whose apertures are so far reduced in size as to materially interfere with the mass movement of the diffusing substance.

A section of the paper is devoted to the application of these new observations to the processes of gaseous and liquid diffusion in living plants, and it is pointed out that the structure of a typical herbaceous leaf illustrates in a striking manner all the physical properties of a multi-perforate septum. Regarded from this point of view it is shown that the stomatic openings and their adjuncts constitute even

a more perfect piece of mechanism than is required for the supply of carbon dioxide for the physiological needs of the plant, and instead of expressing surprise at the comparatively large amount of the gas which an assimilating leaf can take in from the air, we must in future rather wonder that the intake is not greater than it actually is.

From data afforded by actual measurements of the various parts of the stomatal apparatus of the sunflower it is shown that an extremely small difference of tension of the carbon dioxide within the leaf, as compared with that in the outer air, will produce a gradient sufficient to account for the observed intake during the most active assimilation.

It is also shown that the large amounts of water-vapour which pass out of the leaf by transpiration are well within the limits of diffusion, and that it is unnecessary to assume anything like mass movement in the outcoming vapour.

The translocation of solid material from cell to cell in the living plant is next considered, especially with reference to this transference, being, at any rate in part, brought about by means of the minute openings in the cell-walls through which the connecting threads of protoplasm pass. Notwithstanding the very small relative sectional area of these perforations they probably exercise an important function in cell-to-cell diffusion, in virtue of their properties as multiperforate septa.

There are two appendices to the paper, one in which a full description is given of a series of experiments on the absorption of carbon dioxide by solutions of caustic alkali from air in movement; the second being devoted to a detailed description of the methods used for accurately determining the carbon dioxide absorbed.

# Contributions to our Knowledge of the Physiology of the Spermatozoa of Ferns.

BY

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## I. CHEMOTAXIS.

**Introduction.** The reaction of free-swimming organisms to chemical stimuli by alteration in the direction of movement is known as *chemotaxis*. This phenomenon was first generally investigated by Pfeffer<sup>1</sup>. Its results, although not recognized<sup>2</sup> as such, had however previously been observed by Stras-

<sup>1</sup> Locomotorische Richtungsbewegungen durch chemische Reize. Untersuchungen aus d. Bot. Inst. zu Tübingen, Bd. i, p. 363, 1884. Ueber chemotaktische Bewegungen von Bacterien, Flagellaten u. Volvocineen. Ibid., Bd. ii, p. 352, 1888.

<sup>2</sup> Pfeffer, Ber. d. D. Bot. Gesellschaft, 1883, p. 524.



burger<sup>1</sup> during the entrance of spermatozoa into the archegonia of Ferns and *Marchantia*, by Hanstein<sup>2</sup> at a similar moment in the case of *Marsilia*, and by Fischer<sup>3</sup> in the approach of the swarmspores of certain Fungi parasitic on Saprolegniae toward their hosts, while Engelmann<sup>4</sup> had determined that the unequal distribution of oxygen conditions the movements of Bacteria, &c. towards air-bubbles or towards a zone in the medium where the oxygen has a particular pressure. The collections of Infusoria and Bacteria at the surface of decaying organic matter observed by Leeuwenhoek, O. F. Müller, Ehrenberg<sup>5</sup> and Cohn<sup>6</sup> were also probably in part at least due to chemotaxis<sup>7</sup>.

Stahl<sup>8</sup> has found that the plasmodia of the Myxomycetes respond to chemotactic stimuli. Massart<sup>9</sup> and Bordet have shown that this is also true of the leucocytes of the blood.

Pfeffer's experiments were made by a method<sup>10</sup> at once ingenious and simple. Capillary glass tubes about 8 mm long and  $\frac{1}{10}$  mm internal diameter are closed at one end in a gas flame and then placed in a watch-glass. The solution to be tested is poured over the tubes and the watch-glass thereupon set under the bell of an air-pump. On reducing

<sup>1</sup> Strasburger, Jahrb. f. wiss. Bot., 1869-70, Bd. vii, pp. 402 and 418.

<sup>2</sup> Hanstein, Jahrb. f. wiss. Bot., 1865-6, Bd. iv, pp. 219 and 220.

<sup>3</sup> Fischer, Jahrb. f. wiss. Bot., 1882, Bd. xiii, p. 303.

<sup>4</sup> Engelmann, Bot. Zeit., 1881, p. 440. Pflüger's Archiv f. Physiologie, 1881, Bd. xxv, p. 288; also Bd. xxvi, p. 541.

<sup>5</sup> Ehrenberg, Die Infusionsthiere als vollkommene Organismen, 1838, pp. 79 and 80.

<sup>6</sup> Cohn, Beiträge zur Biologie d. Pflanzen, 1872, Bd. i, Heft 2, p. 142. The observations of Leeuwenhoek and O. F. Müller are here cited.

<sup>7</sup> Note.—The collecting of spermatozoa around the egg-cells of *Fucus* which was observed by Thuret (Annales d. sci. nat., 1854, 4<sup>e</sup> sér., tome ii, p. 210) may also be a chemotactic phenomenon. Strasburger's observations appear to support this supposition (Das bot. Practicum, 2. Aufl., 1887, p. 402). Bordet however denies that the spermatozoa are chemotactic (Contribution à l'étude de l'irritabilité des spermatozoïdes chez les Fucacées, Bull. de l'Acad. Belgique, 3<sup>e</sup> sér., tome xxvii, 1894, p. 889).

<sup>8</sup> Stahl, Zur Biologie der Myxomyceten, Bot. Zeit., 1884, p. 165.

<sup>9</sup> Massart et Bordet, Recherches sur l'irritabilité des Leucocytes, Soc. royale des sc. méd. et nat. de Bruxelles, 1890. See also Gabritchsky, Sur les propriétés chimotactiques des Leucocytes, Ann. de l'Inst. Pasteur, 1890, p. 346.

<sup>10</sup> Loc. cit., Bd. i, p. 367.

the pressure of the air the tubes become partly filled with the liquid. A tube is then removed from the solution, washed by dipping in water and placed under the coverglass of a preparation in which spermatozoa or Bacteria, &c., are swimming. If the solution attracts the organisms they collect in the tube, if it repels them they refuse to enter, while if it does not exert a directive stimulus they move in and out of the tube with apparent indifference. This method has been used by Massart, Voegler, Miyoshi and myself.

Pfeffer's researches were made upon spermatozoa (particularly those of Ferns), swarmspores, gametes, Bacteria, Flagellata, Volvocinia and other Infusoria. He found that as a rule when solutions of neutral substances are sufficiently concentrated they repel the organisms, and was at first of the opinion that the repulsion is simply due to a mechanical<sup>1</sup> (osmotic) stimulus. Having, however, found exceptions in glycerine, peptone and alcohol in addition to acids and alkalis he finally<sup>2</sup> attributed the repulsion chiefly, if not entirely, to the chemical qualities of the solutions. Massart<sup>3</sup> has since made a special study of the action of concentrated neutral substances, and has come to Pfeffer's earlier conclusion that the repulsion is usually an osmotic effect. The reaction to the mechanical stimulus is known as *tonotaxis* or *osmotaxis*. Massart made investigations upon a variety of subjects including Bacteria, marine organisms, the muscles of frogs and human eyes. He came to the conclusion that the repulsion of Bacteria, &c. from neutral salts (when this takes place) is solely due to a negative tonotactic stimulus. Some organisms, however, e.g. *Bacterium termo*, appear to be quite insensitive to the effect of concentration.

The relation between stimulus and reaction for chemotaxis has been determined by Pfeffer<sup>4</sup> to be that given in Weber's Law. Although this law has been verified<sup>5</sup> for a tonotactic

<sup>1</sup> Pfeffer, loc. cit., Bd. i, pp. 455, 480.

<sup>2</sup> Bd. ii, pp. 623, 624, 659.

<sup>3</sup> Massart, Sensibilité et adaptation des organismes à la concentration des solutions salines, Arch. de Biologie, t. ix, 1889.

<sup>4</sup> Loc. cit., Bd. i, pp. 395-408.

<sup>5</sup> Rysselberghé, Réactions osmotiques des cellules végétales à la concentration

phenomenon of the cells of higher plants in which the reaction consists of a change of concentration of the cell-sap, this is not yet the case for the tonotaxis of free-swimming organisms. Stange<sup>1</sup> has shown that the zoospores of some Saprolegniaceae are probably attracted to the dead animal bodies, upon which they settle and develop, by the diffusing phosphates. Miyoshi<sup>2</sup> has discovered the interesting fact that the sulphur-bacteria are strongly attracted by solutions of hydrogen sulphide.

Miyoshi<sup>3</sup> has also investigated the phenomenon of chemotropism. He has demonstrated that Fungi penetrate various membranes in response to a chemical stimulus. I<sup>4</sup> have shown that this in general is not the case with Bacteria. Researches upon the chemotropism of pollen-tubes have been made by Molisch<sup>5</sup>, Miyoshi<sup>6</sup> and Lidforss<sup>7</sup>. The tubes are attracted less strongly by sugars and very strongly by certain proteids.

The recent and remarkable papers of Jennings<sup>8</sup> have thrown

du milieu. Mémoires couronnés et autres Mémoires publiés par l'Acad. roy. de Belgique, 1899, t. lviii, p. 1.

<sup>1</sup> Stange, Ueber chemotactische Reizbewegungen. Bot. Zeit., Bd. xlviii, 1890, p. 107, &c.

<sup>2</sup> Miyoshi, Studien über die Schwefelrasenbildung u. die Schwefelbakterien der Thermen von Yumoto bei Nikko. Journ. of the Coll. of Sci., Univers. Tōkyō, vol. x, Pt. 2, 1897.

<sup>3</sup> Miyoshi, Die Durchbohrung von Membranen durch Pilzfaden. Jahrb. f. wiss. Bot., Bd. xxviii, 1895.

<sup>4</sup> Buller, Die Wirkung von Bakterien auf tote Zellen. Dissertation, Leipzig, 1899.

<sup>5</sup> Molisch, Ueber die Ursache der Wachstumsrichtungen bei Pollenschläuchen. Sitzungsber. der Kais. Acad. d. Wiss. in Wien, 1889 and 1893.

<sup>6</sup> Miyoshi, Ueber Reizbewegungen der Pollenschläuche. Flora, Bd. lxxviii, 1894, p. 76.

<sup>7</sup> Lidforss, Ueber den Chemotropismus der Pollenschläuche. Ber. d. D. Bot. Gesell., 1895, Bd. xvii, p. 236.

<sup>8</sup> Jennings, Studies on the reactions to stimuli in unicellular organisms:

- (1) Reactions to chemical, osmotic, and mechanical stimuli in the Ciliate Infusoria, Journ. of Physiology, vol. xxi, 1897, p. 258.
- (2) The mechanism of the motor reactions of Paramecium, Amer. Journ. of Physiology, vol. ii, May, 1899, p. 311.
- (3) Reactions to localized stimuli in Spirostomum and Stentor, American Naturalist, vol. xxxiii, May, 1899, p. 373.



considerable light upon the manner in which the Ciliata and Flagellata respond to chemotactic stimuli. It appears that many of these organisms have a set of reflex movements which can be started by a variety of stimuli such as contact, heat or cold, certain salt solutions, acids, &c. Although no attention is paid to the position of objects and heat-centres, or to the direction of diffusion of dissolved substances setting up the reflex actions, the nature of the latter is such that the organisms move away from the source of stimulation. The same set of reflex actions is also able to cause an 'attraction' toward solutions, &c. Such an 'attraction' is, however, only a particular case of a repulsion. While the majority of organisms investigated by Jennings appear to be chemotactic in the above manner, some were found apparently insensible to chemotactic stimuli. It is certain that in chemotropism the organisms concerned seek to grow along diffusion-lines. The existence of free-swimming organisms which are chemotactic in the sense that they move (directly or screw-wise) along diffusion-lines, although sufficiently probable, is not at present definitely known.

Among the many interesting results of Pfeffer's researches was the discovery<sup>1</sup> that the spermatozoa of the Ferns are strongly attracted by malic acid and its salts. A solution of 0.001 gm per cent. is sufficient to cause a chemotactic reaction. A large number of other substances<sup>2</sup> were tried but only the nearly related maleic acid<sup>3</sup> (as a sodium salt) was found to attract, while attraction by sodiummonobromsuccinate<sup>4</sup> remained doubtful. All the other substances tested, including those most widely found in plant-cells appeared to exert no attractive stimulus whatever. The possibility<sup>5</sup> of the discovery of other substances which attract was, however, ad-

(4) Laws of chemotaxis in *Paramecium*, Amer. Journ. of Physiology, May, 1899, p. 355.

(5) On the movements and motor reflexes of the Flagellata and Ciliata, Amer. Journ. of Physiology, vol. iii, Jan. 1900, p. 229.

<sup>1</sup> Pfeffer, loc. cit., Bd. i.

<sup>2</sup> Loc. cit., Bd. i, pp. 411-13.

<sup>3</sup> Loc. cit., Bd. i, p. 382.

<sup>4</sup> Loc. cit., Bd. ii, p. 655.

<sup>5</sup> Loc. cit., Bd. i, p. 412.

mitted. Sodium maleate was determined to give a much weaker stimulus<sup>1</sup> than sodium malate. It was also pointed out that it is doubtful if maleic acid and its salts occur in plant-cells while on the other hand malic acid is often abundant in cell-sap, and was shown to occur in a decoction<sup>2</sup> of prothallia.

As a result of his work Pfeffer came to the well-known conclusion<sup>3</sup> that it is most highly probable that on the bursting of the archegonia malic acid is the substance liberated which attracts the spermatozoa to the oospheres.

Voegler<sup>4</sup> has since determined that malic acid, sodium, potassium, ammonium, magnesium and calcium malates give approximately equal attractions. He has also repeated those experiments of Pfeffer according to which the diethylester<sup>5</sup> of malic acid appears to act indifferently.

During some work upon the spermatozoa of Ferns I have discovered that a number of other substances beside malic acid exert an attractive stimulus. These include organic and inorganic salts (e.g. those of potassium, tartrates, oxalates and phosphates) commonly found in cell-sap. I have compared the stimulus given by about forty different substances, with the remarkable result that the strongest attraction has been found to be given by malic acid and its salts.

The negative results of Pfeffer appear to be due to the fact that his solutions of organic salts<sup>6</sup> were not sufficiently concentrated, while conclusions were drawn concerning the stimulus given by inorganic salts<sup>7</sup> from the effect observed with mix-

<sup>1</sup> Loc. cit., Bd. i, compare pp. 379-81 and p. 382.

<sup>2</sup> Loc. cit., Bd. i, p. 417.

<sup>3</sup> Loc. cit., Bd. i, p. 417 among others. This conclusion may be received 'mit einer an Gewissheit grenzenden Wahrscheinlichkeit' that it is true.

<sup>4</sup> Voegler, Beiträge zur Kenntniss der Reizerscheinungen. Bot. Zeit., Jahrg. xlix, 1891, p. 659.

<sup>5</sup> Voegler, loc. cit., p. 659. Pfeffer, loc. cit., Bd. ii, p. 655.

<sup>6</sup> Pfeffer, loc. cit., Bd. i, p. 411. The concentrations used were between 0.05 and 0.15 gm %<sub>o</sub>. Spermatozoa are either indifferent to or but very slightly attracted by these solutions.

<sup>7</sup> Loc. cit., Bd. i, p. 410. Solutions were made from the ashes of an extraction from grass. The concentration is not stated. The ammonium nitrate added in

tures. The oversight was rendered all the easier by the relatively strong attraction of malic acid. It was indeed when experimenting with five gm per cent. potassium nitrate that I discovered an attraction by this substance, and was thereby led to make further investigations.

As a result of my work several of Pfeffer's conclusions must undergo modification. Considerable additional probability seems, however, to be given to the supposition that malic acid (in the form of a salt) plays the chief rôle in the attraction of spermatozoa into the archegonia of Fern-prothallia.

When the work was near completion I came to the conclusion that light might be thrown upon the results by an application to them of the electrolytic theory of dissociation of solutions.

**Material and Methods.** The spermatozoa employed were exclusively those from the prothallia of *Gymnogramme Martensii*. Leaves of this Fern were plucked and allowed to dry on sheets of paper. The spores so collected were then sown in pots on a substratum of peat mixed with a little sand and soil. The pots were set in a glass case (about  $3 \times 2 \times 1$  ft.) which was situated in a moderately warmed greenhouse and shaded from the direct rays of the sun by shrubs. The air in the case was kept saturated with water vapour.

The spores were usually sown thickly together. When this was so, the prothallia developed a relatively large number of small antheridia. When the spores had been more scattered the prothallia became larger, heartshaped and with relatively less numerous although larger antheridia. The smaller antheridia contained fewer spermatozoa, sometimes only eight or twelve, while the largest contained as many as sixty-four.

When a pot was found to contain prothallia which had produced sufficient antheridia it was brought into the laboratory and placed under a large bell-jar, which was manipulated in such a way that the prothallia continued to grow in relatively dry air. This appears to enable the antheridia to

one case would help to obscure the effect, for this substance does not attract. (Vide infra, Table I.)



burst more readily and in greater number when the prothallia are placed in water.

In making the solutions of salt to be tested six concentrations of potassium nitrate were used as standards, namely 1,  $\frac{1}{10}$ ,  $\frac{1}{100}$ ,  $\frac{1}{1000}$ ,  $\frac{1}{10000}$ , and  $\frac{1}{100000}$  gram-molecules<sup>1</sup> of this substance dissolved in 1000 ccs water. Since the molecular weight of potassium nitrate is 101 the solutions were respectively 10.1, 1.01, 0.1, 0.01, 0.001 and 0.0001 gm per cent.

The solutions of the other substances were made isotonic with the six standards. As a consequence the solutions in any one vertical row of the Tables I, II, and III taken together are supposed to have an equal attraction<sup>2</sup> for water.

In calculating the solutions required to be isotonic with the standards the isotonic coefficients of de Vries were employed. De Vries<sup>3</sup> has shown that the attraction for water of any substance in solution depends partly upon the structure of the molecules. He proved by means of careful and ingenious experiments upon plant-cells that if the plasmolysing power of a molecule of potassium nitrate in a weak solution be supposed equal to 3, then the plasmolysing power per molecule of any other substance investigated (also in a weak solution) is approximately equal to either 2, 3, 4 or 5. De Vries has called these numbers *isotonic coefficients*.

Substances in Table I, e.g. sodium chloride, have the isotonic coeff. 3, those in Table II, e.g. potassium sulphate, the coeff. 4, while for substances in Table III, e.g. cane-sugar, the coeff. is 2. This being so, it is obvious that to make solutions of sodium chloride, potassium sulphate and cane-sugar isotonic with a solution of potassium nitrate containing  $x$  molecules of the salt it is necessary to take  $\frac{3}{2}x$  mols sodium chloride,  $\frac{3}{4}x$  mols potassium sulphate, and  $\frac{3}{2}x$  mols cane-sugar.

<sup>1</sup> Note.—Instead of writing 1 gram-molecule in 1000 ccs the shorter expression 1 mol will be subsequently employed in this paper. Cf. Grundriss der allgemeinen Chemie, Ostwald, 3. Aufl., p. 70.

<sup>2</sup> Expressed otherwise as osmotic force or plasmolysing power.

<sup>3</sup> Hugo de Vries, Eine Methode zur Analyse der Turgorkraft, Jahrb. für wiss. Bot., Bd. xiv, 1884.

As an illustration of this method of calculation let us suppose it required to make solutions of the three salts just mentioned each isotonic with  $\frac{1}{10}$  mol (approx. 1%) potassium nitrate. The molecular weights of sodium chloride, potassium sulphate, and cane-sugar are respectively 58.5, 174.2, and 342. One-tenth of the gram-molecules are respectively 5.85, 17.42, and 34.2 grams. We shall therefore require  $\frac{3}{2} \times 5.85$  gm sodium chloride,  $\frac{3}{4} \times 17.42$  gm potassium sulphate, and  $\frac{3}{2} \times 34.2$  gm cane-sugar dissolved in 1000 ccs or more simply 0.585, 2.3, and 5.1 gm % of the three substances respectively.

The above method of calculating isotonic solutions gives approximately accurate results only when the concentrations are neither very high nor very low. De Vries has given the higher<sup>1</sup> limit as 3 gm % potassium nitrate (approx.  $\frac{3}{10}$  mol). Another and much more accurate method<sup>2</sup> is based upon our knowledge of the specific molecular conductivity of electricity in solutions at different concentrations. For my purpose, however, as will be clear from the conclusions drawn from the results, the method of de Vries sufficed.

With regard to the chemical aspect of the solutions used it should be observed that in a vertical column of any one table the solutions are isomolecular. In a vertical row of all three tables taken together, however, the solutions in Table I contain more molecules<sup>3</sup> than those in II and less than those in III. The proportionate number of molecules may thus be represented, Table I:II:III::100:75:150.

When making experiments upon the length of life of the spermatozoa I found that they swarmed about three times as long in hanging drops of tap-water as in similar drops of distilled water. Both Pfeffer<sup>4</sup> and Voegler<sup>5</sup> have remarked

<sup>1</sup> De Vries, loc. cit., p. 535.

<sup>2</sup> For an application of this method to physiological work and a comparison of its results and those given by the method of de Vries see the paper of Rysselberghe, 1899, loc. cit.

<sup>3</sup> i. e. taking no account of their dissociation.

<sup>4</sup> Pfeffer, loc. cit., Bd. i, p. 368, Anm. 3.

<sup>5</sup> Voegler, Bot. Zeit., 1891, p. 645.

upon the injurious effects of distilled water. Extremes of heat and exhaustion due to age have been shown<sup>1</sup> to diminish the sensitiveness of the spermatozoa to chemotactic stimuli. It is, therefore, almost certain that a similar diminution in sensitiveness is caused by an unfavourable medium for swarming. On these physiological grounds, therefore, in all my experiments tap-water was used as the medium into which the spermatozoa were liberated and distilled water avoided. Tap-water is, however, a weak salt solution. That used contained about 0.014 gm % mixed salts<sup>2</sup>. All the solutions tested in the capillary tubes were also made with tap-water. The result of this was that the difference in the medium within and without a tube during an experiment was solely due to the extra substance required to be tested<sup>3</sup>.

The prothallia before use were carefully washed in tap-water with the aid of a brush. The coverglasses, usually 12 mm<sup>2</sup>, were supported by the drops of water and the prothallia. Strips of paper were thus avoided. Each experiment was repeated with several tubes. A general account of the method of use of capillary tubes was given in the introduction. For further details reference may be had to the papers of Pfeffer.

In giving the formulae and molecular weights of substances in the Tables (2nd and 3rd columns) the water of crystallization has not been stated. The requisite allowance was, however, made when the salts were weighed. The salts and other substances tested were dried with the necessary caution before use.

**Results.** The first three Tables contain a statement of

<sup>1</sup> Voegler, loc. cit., p. 647, also p. 659.

<sup>2</sup> The average of two analyses made in March, 1896, and July, 1894, kindly supplied me by Prof. Hofmann of the Institute for Hygiene at Leipzig. The water used was practically neutral in reaction to litmus.

<sup>3</sup> The criticism may be offered that in making the solutions with tap-water reactions would in some cases take place which might affect the results. This is true. If, however, the solutions were made with the distilled water, on diffusing out of the tubes reactions would take place with the tap-water outside. I am inclined to think that the conditions under which the spermatozoa swim in nature



results. The explanation of the terms employed is as follows:—

A=marked attraction.

R=repulsion.

a =weak attraction.

o =indifferent.

rA=repulsion at first, afterwards strong attraction.

N=spermatozoa did not collect in the tubes.

— =no experiment made owing to low solubility or acidity of substance.

The third column contains the molecular weights of the salts employed.

When a tube is filled with a solution of a neutral salt which gives a marked attraction (A), the concentration being  $\frac{1}{10}$  mol, a few minutes after the beginning of the experiment the spermatozoa are seen collecting at the mouth of the tube and also just inside in considerable numbers. In this diffusion-zone the spermatozoa move backward and forward for some seconds or minutes, being alternately attracted and repelled. It is only after repulsion from a certain diffusion-zone within the tube has taken place several times that the majority of spermatozoa finally enter deeply into the solution. Within one minute after penetrating down the tube they come to rest owing to the withdrawal of water from their protoplasm by the salt solution. Being heavier than the solution they quickly sink to the bottom of the tube, where they may be easily counted. The collections of spermatozoa amount to 100–1000, according to the number liberated from the prothallia and the specific action of each salt.

When a neutral salt which attracts is tested at a concentration isotonic with  $\frac{1}{100}$  mol potass. nitrate a weak attraction (a) usually takes place. The spermatozoa enter the tube without hesitation at the mouth, the repulsion described above not making itself evident. The attraction, however, only lasts a few minutes. The spermatozoa collect in smaller numbers. They continue their motion in the tube, and when

are much more like those here employed than would have been the case if distilled water had been used.

the concentration of the solution has so far sunk that attraction no longer takes place, they may move out again. In several cases I could detect a weak attraction only after repeating the experiments a considerable number of times.

Malic acid and its neutral salts have a strong attraction (denoted by A) at a concentration isotonic with  $\frac{1}{1000}$  mol potassium nitrate. There is no hesitation in entering the tubes, where the spermatozoa continue to swim for some minutes. In a successful experiment several hundred organisms may be collected.

When a solution acts indifferently the spermatozoa enter the tube occasionally, but do not collect there. If uninjured by the solution they move as readily out of the tube as into it. Indifference may be well observed when the tube contains tap-water. In making the experiments this control was often employed.

A repulsion denoted by R is always very definite. The substances (two acids and an acid salt) found to give such a repulsion also attract at lower concentrations. If a tube contains one of these repellent acid substances at sufficient concentration the spermatozoa are attracted towards the mouth. On reaching a diffusion-zone which causes repulsion the spermatozoa reverse their direction of motion and move rapidly away from the mouth of the tube in a fairly straight line, and thus reach a zone which no longer repels. Here the normal more or less circular direction of motion is resumed. The flight from a repellent zone is easily recognized. Owing to the attraction at low concentrations and repulsion at higher the spermatozoa may collect in a ring outside the tube. The ring indicates the position of a diffusion-zone at the intermediate concentration. During an experiment, owing to diffusion, the zone gradually approaches the mouth of the tube which it may finally enter.

It must be pointed out that the repulsion R just described differs considerably from the repulsion which makes itself evident in the case of a marked attraction (A) by a neutral

salt. The repulsion R is protective, for it prevents the spermatozoa entering too deeply into the acid substances and thus becoming injured. The slight repulsion which occurs during an attraction by a neutral salt is, however, not protective, for the spermatozoa finally enter too deeply into the tube, where they are immediately brought to rest from loss of water.

The repulsion R from the acid substances is undoubtedly chemotactic. If it were tonotactic, sugar, asparagin and neutral salts should repel when tested at concentrations isotonic with repellent solutions of the acid substances. A comparison of the data given in the tables makes it evident that this is not the case. The repulsive effect which is to be observed during a marked attraction by a neutral salt may on the other hand be tonotactic. If so the tonotactic repulsion is very slight and easily overcome by the chemotactic attraction.

The term N has been used to indicate that no collection takes place in the tubes at the concentrations isotonic with 1 mol potassium nitrate. A collection in these cases appears to be mechanically impossible. If a substance attracts, e.g. potassium nitrate, the spermatozoa may collect in the diffusion-zones outside the mouth of the tube. On approaching the mouth, however, the spermatozoa come to rest owing to the withdrawal of water from their protoplasm. They cannot at best penetrate more than one diameter into a tube, and when they do so are slowly carried out again by currents<sup>1</sup>. Solutions isotonic with 1 mol potassium nitrate are comparatively heavy. In consequence they fall with apparent rapidity out of the tubes. This phenomenon prevents the collections of spermatozoa in the less concentrated diffusion-zones from being clear. With a solution of  $\frac{1}{2}$  mol potassium nitrate the collection outside the tube may be definitely observed. At the beginning of the experiment the spermatozoa may penetrate 2-3 diameters down the tube before

<sup>1</sup> The outgoing stream on the bottom of the tube and into which the spermatozoa fall after coming to rest.



coming to rest, but as a rule are carried out again by currents.

It has been remarked that when spermatozoa pass down a tube containing a neutral salt isotonic with  $\frac{1}{10}$  mol potassium nitrate they come to rest within one minute. They do not penetrate more than half-way down a tube and usually less. With an isotonic solution of glycerine or alcohol the spermatozoa which have entered a tube accidentally may swim down to the bottom and for some minutes before coming to rest. On entering the alcohol the spermatozoa continue to swim as rapidly as in water. On entering the glycerine, their motion is much less rapid. If a solution of glycerine isotonic with 1 mol potassium nitrate is in a tube, spermatozoa accidentally entering penetrate somewhat further and move longer than in a similar experiment with the latter salt. With an isotonic solution of alcohol (6.9 grams per cent.) the contrast with the neutral salts is very striking. On entering a tube a spermatozoon may move down to the bottom, and for one to five minutes. In a similar experiment with potassium nitrate, as already stated, the spermatozoa at best only penetrate a distance equal to about one diameter and come to rest immediately.

The explanation of the above differences depends upon the rate of penetration of the substances in question through living membranes. Neutral salts, sugar, &c., penetrate extremely slowly or not at all, glycerine<sup>1</sup> comparatively quickly, and alcohol<sup>2</sup> very quickly. A spermatozoon comes to rest when a certain amount of water is withdrawn from it. It seems to follow, therefore, that the distance a spermatozoon may penetrate down a tube depends partly upon the specific rate of penetration through living membranes of the substance tested.

<sup>1</sup> De Vries, Ueber den isoton. Coeff. des Glycerins. *Bot. Zeit.*, 1888, nos. 15 and 16.

<sup>2</sup> Overton, Ueber die allgemeinen osmotischen Eigenschaften der Zelle, ihre vermuthlichen Ursachen und ihre Bedeutung für die Physiologie. *Vierteljahrsschrift der Naturforschenden Gesell. in Zürich*, xlv, 1899.

From the facts embodied in the tables it is evident that in addition to malic acid and its salts, a considerable number of organic and inorganic salts often occurring in plant cell-sap also attract spermatozoa. All the organic salts tested were found to attract. These include tartrates, potassium oxalate, potassium acetate, and sodium formate. Among the attracting inorganic salts are phosphates, sulphates, potassium nitrate, and potassium chloride.

TABLE I. *Isotonic coeff.* = 3.

Standard solutions of $\left\{ \begin{array}{l} \text{in parts of a mol} \\ \text{Potassium nitrate} \end{array} \right.$ in grams per cent.			1 10.1	$\frac{1}{10}$ 1.01	$\frac{1}{100}$ 0.1	$\frac{1}{1000}$ 0.01	$\frac{1}{10000}$ 0.001	$\frac{1}{100000}$ 0.0001
Ammonium hydrogen malate	$\text{NH}_4\text{C}_4\text{H}_5\text{O}_5$	151	R	R	R	rA	a	o
Potassium nitrate	$\text{KNO}_3$	101	N	A	a	o	o	o
Sodium nitrate	$\text{NaNO}_3$	85	N	o	o	o	o	o
Lithium nitrate	$\text{LiNO}_3$	69	N	o	o	o	o	o
Ammonium nitrate	$\text{NH}_4\text{NO}_3$	80	N	o	o	o	o	o
Potassium chloride	$\text{KCl}$	74.6	N	A	o	o	o	o
Sodium chloride	$\text{NaCl}$	58.5	N	o	o	o	o	o
Ammonium chloride	$\text{NH}_4\text{Cl}$	53.5	N	o	o	o	o	o
Rubidium chloride	$\text{RbCl}$	120.7	N	A	o	o	o	o
Potassium bromide	$\text{KBr}$	119.1	N	A	a?	o	o	o
Potassium iodide	$\text{KI}$	166.1	N	A	o?	o	o	o
Potassium chlorate	$\text{KClO}_3$	122.6	—	A	o	o	o	o
Sodium formate	$\text{NaCHO}_2$	68	N	A	o	o	o	o
Potassium acetate	$\text{KC}_2\text{H}_3\text{O}_2$	98.14	N	A	a	o	o	o

Organic substances which act indifferently are grape-sugar, cane-sugar, lactose, amyloextrin, glycerine, alcohol, asparagin, and urea. Inorganic salts not appreciably attracting are the chlorides and nitrates of sodium, ammonium, and calcium, and also lithium nitrate.

Of the four free acids which seem to be the most widely found in cell-sap, namely malic, oxalic, tartaric and citric, only malic acid attracts.

In comparing the effect of the stimulus given by different substances, one at once notices the somewhat striking fact that compounds containing the negative radicle of malic acid attract much more strongly than any other tested. In each table is to be found a substance with the acid radicle in question. In Table I it will be noticed that whereas the malate attracts strongly at the concentration  $\frac{1}{1000}$  mol,

TABLE II. *Isotonic coeff.* = 4.

Standard solutions of <i>Potassium nitrate</i> { <i>in parts of a mol in grams per cent.</i>			$\frac{1}{10 \cdot 1}$	$\frac{1}{10}$ $\frac{1}{1 \cdot 01}$	$\frac{1}{100}$ $\frac{1}{0 \cdot 1}$	$\frac{1}{1000}$ $\frac{1}{0 \cdot 01}$	$\frac{1}{10000}$ $\frac{1}{0 \cdot 001}$	$\frac{1}{100000}$ $\frac{1}{0 \cdot 0001}$
Sodium malate	$\text{Na}_2\text{C}_4\text{H}_4\text{O}_5$	178.12	N	A	A	A	a	o
Potassium tartrate	$\text{K}_2\text{C}_4\text{H}_4\text{O}_6$	226.2	N	A	a	o	o	o
Sodium tartrate	$\text{Na}_2\text{C}_4\text{H}_4\text{O}_6$	194.1	N	A	a	o	o	o
Sodium-potass. tartrate	$\text{KNaC}_4\text{H}_4\text{O}_6$	210.2	N	A	a	o	o	o
Potassium oxalate	$\text{K}_2\text{C}_2\text{O}_4$	166.2	N	A	a	o	o	o
Potassium phosphate	$\text{K}_2\text{HPO}_4$	174.2	N	A	a	o	o	o
Sodium phosphate	$\text{Na}_2\text{HPO}_4$	142	N	A	a	o	o	o
Ammoniumphosphate	$\text{Am}_2\text{HPO}_4$	132	N	A	a	o	o	o
Potassium sulphate	$\text{K}_2\text{SO}_4$	174.2	N	A	a	o	o	o
Sodium sulphate	$\text{Na}_2\text{SO}_4$	142	N	A	a?	o	o	o
Ammonium sulphate	$\text{Am}_2\text{SO}_4$	132	N	A	a	o	o	o
Caesium sulphate	$\text{Cs}_2\text{SO}_4$	362	N	A	o	o	o	o
Sodium sulphite	$\text{Na}_2\text{SO}_3$	126	N	A	a	o	o	o
Sodium thiosulphate	$\text{Na}_2\text{S}_2\text{O}_3$	158	N	A	a?	o	o	o
Calcium nitrate	$\text{Ca}(\text{NO}_3)_2$	164	N	o	o	o	o	o
Calcium chloride	$\text{CaCl}_2$	110.9	N	o	o	o	o	o
Potassium carbonate	$\text{K}_2\text{CO}_3$	138.2	N	N	o?	o	o	o

and also, although very weakly<sup>1</sup>, at  $\frac{1}{100000}$ , the other salts at these concentrations do not give an observable reaction. A similar statement is true of Table II, and also of Table III if maleic acid be excepted. Maleic acid, however, only gives a very weak attraction at a concentration isomolecular with

<sup>1</sup> Compare Pfeffer, loc. cit., Bd. i, p. 379, and Voegler, Bot. Zeit., 1891, No. 40, p. 659.



$\frac{3}{2} \times \frac{1}{10000}$  mol malic acid, and is apparently indifferent at a solution one-tenth as strong which in the case of malic acid gives a just appreciable reaction.

With potassium nitrate I could detect no attraction at 0.05 gm per cent., whereas there is a slight one at 0.1 per cent. The concentration necessary to give a just appreciable reaction lies then between  $\frac{5.0}{10000}$  and  $\frac{1.00}{10000}$  mol. Malic acid gives a just observable reaction at 0.001 gm per cent.

TABLE III. *Isotonic coeff.* = 2.

Standard solutions of Potassium nitrate		in parts of a mol in grams per cent.	1 10.1	$\frac{1}{10}$ 1.01	$\frac{1}{100}$ 0.1	$\frac{1}{1000}$ 0.01	$\frac{1}{10000}$ 0.001	$\frac{1}{100000}$ 0.0001
Cane sugar	$C_{12}H_{22}O_{11}$	342	N	o	o	o	o	o
Grape sugar	$C_6H_{12}O_6$	180	N	o	o	o	o	o
Amylodextrin	$(C_{12}H_{20}O_{10})_3$	972	—	o	o	o	o	o
Lactose	$C_{12}H_{22}O_{11}$	342	—	o	o	o	o	o
Glycerine	$C_3H_5(OH)_3$	92	o	o	o	o	o	o
Ethyl alcohol	$C_2H_6O$	46	o	o	o	o	o	o
Asparagin	$C_4H_4O_3(NH_2)_2$	132	—	o	o	o	o	o
Urea	$CO(NH_2)_2$	60	N?	o	o	o	o	o
Malic acid	$C_4H_6O_5$	134	R	R	R	A	a	o
Maleic acid	$C_4H_4O_4$	116	R	R	R	a	o	o
Tartaric acid	$C_4H_6O_6$	150	—	—	o?	o	o	o
Citric acid	$C_6H_8O_7$	192	—	—	o?	o	o	o
Oxalic acid	$C_2H_2O_4$	90	—	—	o?	o	o	o
Magnesium sulphate	$MgSO_4$	120	N	A	a	o	o	o

or at a concentration isomolecular with one less than  $\frac{1}{10000}$  mol potassium nitrate. A rough estimate gives us, therefore, the result that malic acid attracts at least fifty times more strongly than potassium nitrate.

For the other salts which attract the concentrations necessary to cause a just appreciable attraction have not been precisely determined. They are, however, near those isomolecular with  $\frac{1}{100}$  mol potassium nitrate, in some cases slightly higher, in most slightly lower. It would seem,

therefore, a moderate estimate if we conclude that, excluding malates and maleates, malic acid attracts the spermatozoa about fifty times more strongly than any of the other attracting substances tested.

As an illustration of the difference in the strength of attraction, it may be remarked that I have repeated the experiments with potassium tartrate with the greatest care on several different occasions, and have never been able to determine the least attraction at  $\frac{3}{4} \times 1000$  mol, while with an isomolecular solution of malic acid a strong attraction is always found to take place.

The biological aspect of the above results is interesting. The facts certainly support the supposition of Pfeffer already mentioned, that a substance containing the negative radicle of malic acid is liberated at the bursting of the archegonia, and serves to attract the spermatozoa to the oospheres. It is not impossible, however, that a supplementary rôle may be played in this phenomenon by other substances or another substance such as a tartrate or an inorganic potassium salt.

There are several arguments against the supposition that it is the free acid which attracts spermatozoa to the archegonia. In the first place, Pfeffer<sup>1</sup> could determine no acid reaction to be given by the liberated cell-sap when the archegonia burst. Secondly, the free acid, even at low concentrations, is very toxic for the spermatozoa, for it shortens their swarm-period considerably. Thus I found that they live about twelve times longer in tap-water than in a solution of tap-water containing 0.01 gm per cent. malic acid. For the purpose of the third argument it is necessary to give the data showing the manner in which the stimulus of malic acid varies with the concentration. In Table IV: R = repulsion, A = marked attraction, a = weaker attraction, R-A = repulsion at first quickly followed by strong attraction, o = indifferent. The solutions are given in grams per cent.

<sup>1</sup> Loc. cit., Bd. i, p. 418.

TABLE IV. *Malic acid.*

Concentration	1.0	0.05	0.04	0.03	0.02	0.01	0.001	0.0005
Stimulus	R	R	R	R-A	A	A	a	o

From the results given in the table it will be noticed that a repulsion takes place at 0.03 gm per cent. Now Pfeffer<sup>1</sup> found that if prothallia are placed in a solution of 0.01 gm per cent. sodium malate the spermatozoa liberated are still just observably attracted into the archegonia. He has also found<sup>2</sup> that if any solution  $x$  of the malate be used outside a capillary tube as a medium in which the spermatozoa swim, then, in order to obtain an observable reaction it is necessary to place in the tube a solution = 30  $x$ . When, therefore, he finds that if the medium contains 0.01 per cent. sodium malate, an attraction<sup>3</sup> into the archegonia still takes place, we are justified in accepting his conclusion that the least concentration of the malate liberated is 0.3 per cent. It may, however, be deduced from Table IV that a solution of the free acid isomolecular with 0.3 per cent. sodium malate strongly repels. It is therefore evident that it is not the free acid which plays the chief rôle in the attraction of spermatozoa into the archegonia. It is much more probably one or more neutral salts.

Pfeffer<sup>4</sup> tested various proteids and sugars (see also Table IV) and was unable to find any which attracted the spermatozoa of Ferns. At present the substances known to attract may be classed as organic acids, organic salts and inorganic salts. The chemotropism of pollen-tubes offers an interesting contrast to the chemotaxis of the spermatozoa. Chemotropic deviations are caused by proteids<sup>5</sup> and sugars, and as yet no salt or acid has been found to attract.

Pollen-tubes pass from the stigma to the ovules by growth. It should not, therefore, be surprising that the food-stuffs—

<sup>1</sup> Loc. cit., Bd. i, p. 418.

<sup>2</sup> Loc. cit., Bd. i, p. 398.

<sup>3</sup> Loc. cit., Bd. i, p. 418.

<sup>4</sup> Bd. i, pp. 412-413.

<sup>5</sup> Lidforss, loc. cit., p. 237.



proteids and sugar—on which the tube lives, are also employed<sup>1</sup> in giving the chemotropic stimulus. On the other hand, the spermatozoa of the Ferns have stored within them sufficient energy to reach the oospheres without the help of any additional food-supply from without. It is therefore not essential that the substance (or substances) liberated from the archegonia should possess a high value as food. Malates might therefore be employed to attract the spermatozoa. The acid radicle of malic acid is present in a decoction<sup>2</sup> of prothallia, and is doubtless a normal product of the metabolism of the cells. This fact helps to explain why in all probability a malate is set free from the archegonia in order to attract the spermatozoa.

**The dissociation theory and chemotaxis.** The theory of electrolytic dissociation of solutions has been successfully employed in estimating the toxic effect of various substances upon higher plants<sup>3</sup>, Bacteria<sup>4</sup>, and Fungi<sup>5</sup>.

The first suggestion that chemotaxis may to a certain extent be elucidated by the dissociation theory is due to Ostwald<sup>6</sup>. This physical chemist explains the fact, discovered by Pfeffer, that the salts of malic acid attract the spermatozoa of Ferns with about equal strength, whereas the diethylester does not attract at all, on the supposition that in the salt solutions the negative radicle is free as an ion, and in this form attracts, while in the ester solution it is not free, this substance being undissociated.

In a recent paper by Jennings<sup>7</sup>, entitled 'The Laws of chemotaxis in *Paramecium*,' an endeavour was made to

<sup>1</sup> Lidforss, loc. cit.

<sup>2</sup> Pfeffer, Bd. i, p. 417.

<sup>3</sup> Kahlenberg and True, On the toxic action of dissolved salts and their electrolytic dissociation. Bot. Gaz., Vol. XXII, 1896, p. 81. Heald, On the toxic effect of dilute solutions of acids and salts upon plants. Ibid., p. 125.

<sup>4</sup> Krönig u. Paul, Die chemischen Grundlagen der Lehre von der Giftwirkung u. Desinfektion. Zeit. f. Hygiene u. Infect., Bd. xxv, 1897, p. 1.

<sup>5</sup> Clark, Electrolytic dissociation and toxic effect. Jour. of Phys. Chem., Vol. III, 1899, p. 263.

<sup>6</sup> Ostwald, Referat, Zur Pharmakologie des Quecksilbers, E. Dreser, Zeit. f. phys. Chem., Bd. xiii, 1894, p. 378.

<sup>7</sup> Jennings, Study IV. loc. cit., May, 1899.

explain the results with sixty-five compounds by the help of a chemical theory. Unfortunately the attempt miscarried, 'owing to a chemical misinterpretation as to the facts which give solutions their characteristic properties.' In a footnote of a subsequent paper<sup>1</sup> Jennings has partially corrected his earlier criticism. The results of his work seem to leave little doubt that the chemotaxis of *Paramecium* follows certain laws to which the dissociation theory gives a clue. The theory has also been applied by Garrey<sup>2</sup> to the chemotaxis of *Chilomonas*.

It must not be assumed that a chemotactic stimulus may be given only by ions. Just as some undissociated substances, such as alcohol, have a toxic action, so one finds undissociated substances giving a chemotactic stimulus. Thus, for instance, Pfeffer found that the <sup>3</sup>spermatozoa of Mosses are attracted by cane-sugar which is not dissociated, and his further work upon Bacteria has shown that a single species, e. g.<sup>4</sup> *Bacterium termo*, is attracted by a number of undissociated substances e. g. grape sugar, dextrin, milk sugar, mannit and asparagin in addition to such highly dissociated substances as sodium chloride, potassium nitrate and potassium sulphate.

In testing the toxic action of solutions it appears to have been determined in some cases at least that even where the substance is highly dissociated the undissociated molecules<sup>5</sup> have a toxic effect. We must therefore admit that when a highly dissociated salt (e. g. potassium nitrate as a 1% solution) attracts spermatozoa, the small percentage of undissociated molecules may have a chemotactic action.

<sup>1</sup> Study, V, On the movements and motor reflexes of the Flagellata and Ciliata. Amer. Jour. of Physiology, Jan., 1900. The footnote on p. 236 contains the above citation.

<sup>2</sup> Garrey, The effect of ions upon the aggregation of Flagellated Infusoria. Amer. Jour. of Physiology, Vol. III, p. 239, Jan., 1900.

Note. The papers of Jennings and Garrey came to my knowledge after this section of my paper was written. It has been altered only in so far as it was thought necessary to acknowledge their work. Garrey's paper appeared six months after my work was brought to a conclusion.

<sup>3</sup> Pfeffer, loc. cit., Bd. i, p. 430.

<sup>4</sup> Ibid., Bd. ii, pp. 603-605.

<sup>5</sup> Clark, loc. cit., pp. 282, 287, &c.

In Tables I and II it will be noticed that, whereas potassium nitrate attracts, the nitrates of sodium, lithium, ammonium and calcium do not, and that while potassium chloride attracts this is not the case with the chlorides of sodium, ammonium and calcium.

The solutions of these salts are ionized, the ions being in each case the negative radicle and the metal. The amount of ionization is considerable. At a concentration isomolecular with  $\frac{1}{10}$  mol potassium nitrate<sup>1</sup>, it is 70–86% of the molecules in solution, while at lower concentrations it is of course still higher. From the fact that the solutions of sodium, ammonium, lithium and calcium nitrates and also sodium, ammonium and calcium chlorides do not attract it would seem justifiable to conclude that neither the ions  $\text{Na}^+$ ,  $\text{NH}_4^+$ ,  $\text{Li}^+$ ,  $\text{Ca}^{++}$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$  nor the whole molecules  $\text{NaCl}$ ,  $\text{NH}_4\text{Cl}$ ,  $\text{CaCl}_2$ ,  $\text{NaNO}_3$ ,  $\text{NH}_4\text{NO}_3$ ,  $\text{LiNO}_3$ ,  $\text{Ca}(\text{NO}_3)_2$  give a positive chemotactic stimulus.

Potassium nitrate and potassium chloride both attract. If, however, the ions  $\text{Cl}^-$  and  $\text{NO}_3^-$  do not attract it is evident that the attraction of these salts is due to the ion  $\text{K}^+$  or possibly to the whole molecules  $\text{KNO}_3$  and  $\text{KCl}$  or to the combined effect of the  $\text{K}^+$  ions and the respective whole molecules. At a concentration of  $\frac{1}{10}$  mol, potassium nitrate is ionized to the extent of approx. 81% and potassium chloride to 86%. Owing to this high dissociation and also to the known chemical activity of the ions in a solution it seems to me that we may neglect the second possibility and assume that it is highly probable that the ion  $\text{K}^+$  gives a positive chemotactic stimulus.

If  $\text{K}^+$  ions attract, it is reasonable to suppose that any other neutral salt of potassium in which the ion  $\text{K}^+$  is present also attracts. So far as my experiments have gone with nine other potassium salts this supposition has been justified. Thus attraction is given by solutions of potassium iodide, bromide, sulphate, chlorate, phosphate ( $\text{K}_2\text{HPO}_4$ ), tartrate, acetate, oxalate and sodium potassium tartrate.

<sup>1</sup> Calculated from the tables in 'Solution and Electrolysis.' Whetham, 1895.



The strongly alkaline potassium carbonate does not attract. This is not surprising on account of the fact that this substance (owing to hydrolysis<sup>1</sup> and consequent formation of  $\text{OH}^-$  ions) exercises a strongly toxic effect upon spermatozoa at a concentration below that probably necessary for the ion  $\text{K}^+$  to give an appreciable attraction.

The following<sup>2</sup> substances give attractions of approximately equal strength: malic acid, ammonium hydrogen malate and the malates of sodium, potassium, ammonium, calcium, barium and magnesium. All these substances are dissociated and each contains the negative radicle of malic acid,  $\text{C}_4\text{H}_4\text{O}_5^-$ . They attract, as already mentioned, much more strongly than any other substances which do not contain the radicle in question. Some of the bases, namely sodium, ammonium and calcium, when occurring as ions in solution, according to my previous argument do not appear to appreciably attract at all. We may therefore conclude that the  $\text{C}_4\text{H}_4\text{O}_5^-$  ions attract.

Although we have good reason to suppose that the ion  $\text{K}^+$  attracts, solutions other than malates containing  $\text{K}^+$  ions cease to attract at the concentration approx.  $\frac{1}{200}$  mol. Potassium malate, however, attracts at  $\frac{1}{10000}$  mol. It appears, therefore, that at this concentration potassium malate owes its attraction entirely to the  $\text{C}_4\text{H}_4\text{O}_5^-$  ions, while the  $\text{K}^+$  ions are practically chemotactically inactive. It is doubtless due to the indifference of all the kations at low concentrations that all malates attract with about equal strength.

If it is the ion  $\text{C}_4\text{H}_4\text{O}_5^-$  of malic acid which is responsible for the attraction of this substance it is no matter for surprise that when this group of atoms is present in an undissociated compound, attraction no longer takes place. This happens in the case of the di-ester of malic acid, as was pointed out by Ostwald. Both Pfeffer<sup>3</sup> and Voegler<sup>4</sup> found that this substance

<sup>1</sup> Ostwald, *Grundlinien der anorganischen Chemie*, 1900, pp. 255, 256.

<sup>2</sup> Compare Tables I, II, and III; Pfeffer, loc. cit., Bd. i, p. 381; and Voegler, loc. cit., p. 659.

<sup>3</sup> Bd. ii, p. 655. (1% and 0.1% solutions were tried.)

<sup>4</sup> Voegler, loc. cit., p. 659.

does not attract the spermatozoa. It is still less astonishing that asparagin, which may be written as an amide-derivative<sup>1</sup> of malic acid, does not attract, for not only is this compound practically undissociated, but the group of atoms which forms the negative radicle of malic acid is no longer present.

Five sulphates have been tested, namely, those of sodium, potassium, ammonium, magnesium and caesium. They all attract. From what has already been said about the ions  $\text{Na}^+$  and  $\text{NH}_4^+$  it follows that we must attribute the attraction of sulphates to the negative radicle. It seems, therefore, very probable that the ion  $\text{SO}_4^-$  attracts.

A similar argument may be applied to the phosphates and tartrates. Their attraction is probably due to the respective anions.

With regard to the ions of other neutral salts, there is not sufficient evidence to draw any very definite conclusions. From the fact, however, that sodium formate, sodium sulphite and sodium thio-sulphate attract, it appears probable that the respective anions give a positive chemotactic stimulus. There is at present no evidence to indicate the action of the ions  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{ClO}_3^-$ ,  $\text{C}_2\text{H}_3\text{O}_2^-$  and  $\text{C}_2\text{O}_4^-$ .

The eight undissociated substances tested namely, cane sugar, grape-sugar, lactose, amyloextrin, alcohol, glycerine, asparagin and urea, agree in not attracting the spermatozoa. The indifference of the carbohydrates is remarkable, in view of the fact that the spermatozoa of the Mosses are attracted by cane-sugar. Undissociated substances may yet be discovered which attract the spermatozoa of Ferns. Such substances will probably not be found among sugar or alcohols, owing to the fact that cane-sugar, grape-sugar, lactose and amyloextrin on the one hand and ethyl-alcohol and glycerine on the other act indifferently.

A comparison may now be made between salts and acids. In Table II it will be seen that sodium malate attracts at the concentrations isotonic with  $\frac{1}{10}$ ,  $\frac{1}{100}$ ,  $\frac{1}{1000}$ , and  $\frac{1}{10000}$  mol

<sup>1</sup> Asparagin is, however, to be regarded as the monamide of amido-succinic acid. Höllemann, *Lehrbuch d. organ. Chemie*, 1899, p. 221.

potassium nitrate. If the concentrations are doubled so that they become isomolecular with the solutions in Table III attraction still takes place at the new concentrations. Sodium malate attracts therefore in solutions isomolecular with  $\frac{3}{2} \times \frac{1}{10}$ ,  $\frac{3}{2} \times \frac{1}{100}$ ,  $\frac{3}{2} \times \frac{1}{1000}$ ,  $\frac{3}{2} \times \frac{1}{10000}$  mol malic acid. The free acid however, while attracting at the two latter concentrations, repels at the two higher. Sodium hydrogen malate and maleic acid behave in a similar manner. Common to these three acid substances in solution is the ion  $H^+$ , whereas in the neutral salts it is absent. One would suppose, therefore, that the ion  $H^+$  is responsible for the repulsion.

If however the ion  $H^+$  is the cause of repulsion, we might also expect to find that other acids such as tartaric, oxalic, and citric repel. This supposition I have not as yet verified. I was able to satisfy myself that these three acids do not attract, but remained in doubt<sup>1</sup> whether they repel or are chemotactically indifferent. According to Pfeffer<sup>2</sup>, however, citric acid repels. From his work with Bacteria, &c., he came to the conclusion<sup>3</sup> that repulsion by acids at sufficient concentration is a general phenomenon. Jennings has found that *Paramecium* is repelled by all acids at the necessary concentration. We have, therefore, considerable support for the supposition that the ion  $H^+$  is the cause of repulsion from acids.

Reference has already been made to the ring-collections which may be obtained in an experiment with a strong solution of malic acid. A ring-collection is due to the fact that malic acid attracts at low concentrations and repels at higher. The part played by the ions may perhaps be explained in the following manner. At 0.03% malic acid (see

<sup>1</sup> At this point in the research the material unfortunately became attacked by a Fungus, and in consequence the spermatozoa available for any given preparation were not numerous enough to decide the question. A repulsion is easy to observe when there is also an attraction because the spermatozoa collect in the intermediate diffusion zone. When there is no such attraction a repulsion must undoubtedly be much more difficult to observe. When Bacteria are used the detection of a repulsion with a substance that does not attract is much easier owing to the enormous number of individuals one may employ.

<sup>2</sup> Pfeffer, loc. cit., Bd. i, p. 387.

<sup>3</sup> Ibid., Bd. ii, p. 659.



Table IV) the attraction of the  $C_4H_4O_5^-$  ions equals the repulsion of the  $H^+$  ions. At concentrations below this, owing to the specific reaction of the spermatozoa, the combined attractive effect of the  $C_4H_4O_5^-$  ions is greater than the combined repulsive effect (if such there is) of the  $H^+$  ions. The spermatozoa, therefore, collect in weak solutions of the acid. At concentrations above 0.03% the combined repulsive effect of the  $H^+$  ions becomes greater than the combined attractive effect of the  $C_4H_4O_5^-$  ions. A repulsion therefore takes place<sup>1</sup>.

Engelmann<sup>2</sup> discovered that Bacteria are attracted by oxygen dissolved in water at a certain pressure and repelled when the pressure exceeds a certain limit. In this case the *same molecules* evidently give a positive or negative chemotactic stimulus according to their concentration.

Rubidium chloride attracts. If my argument that the  $Cl^-$  ion is inactive is correct, it is very probable that the  $Rb^+$  ions are responsible for the attraction. The ions  $Rb^+$  and  $K^+$  attract, while the ions  $Na^+$ ,  $NH_4^+$  and  $Li^+$  do not. The grouping of these elements agrees with that given in the Periodic Law.

It is now necessary to make some remarks upon the nature of an 'attraction.' Owing to the fact that the papers of Jennings (loc. cit.) came to my notice when too late, I made no special study of the motor reactions of the spermatozoa, but assumed that an attraction is due to a sensitiveness and consequent reaction on the part of the organisms to a gradation in concentration along the surface or in the substance of their bodies. If this theory is correct an attraction is directly caused by a solution in which the spermatozoa collect. There is but one external stimulus. It is this, which my application of the dissociation theory may concern. The stimulus just suggested is, however, according to the observations of Jennings<sup>3</sup>, not the cause of chemotactic

<sup>1</sup> This explanation may be somewhat more complicated, but not different in nature, owing to the fact that malic acid dissociates in stages.

<sup>2</sup> Engelmann, *Zur Biologie der Schizomyceten*. Pflüger's Archiv f. Physiologie, 1881, Bd. xxvi, p. 541.

<sup>3</sup> Jennings, *Studies II and V*, loc. cit.

movements in the Flagellata and Ciliata. A collection of these organisms in a drop introduced into the medium where they are swimming takes place as follows. (1) The organisms enter the drop accidentally, (2) the drop acts upon the organisms, (3) in consequence, when they accidentally approach their previous medium it repels them. Here there are two distinct kinds of action. The drop is responsible for the first, and the previous outer medium for the second. If the motor reactions of the spermatozoa are of the same nature as those of the Ciliata and Flagellata it must be understood that when, in the application of the dissociation theory, ions or molecules are said to 'attract,' this means that they give rise to the first of the two actions.

In conclusion it must be pointed out that in discussing the attraction of neutral salts I have been unable to state whether the whole molecules play any chemotactic rôle. The omission is, however, rendered less serious by two considerations. Firstly, owing to the high dissociation at the low concentrations used, the number of whole molecules in any unit volume of a solution is usually very much less than the number of ions. Secondly, the ions in any solution are supposed by chemists<sup>1</sup> to be responsible for its chemical reactions. The assumption that the attraction by a neutral salt is practically due to one or both ions is therefore to a considerable extent justified. Since, however, in the work upon toxicity (*loc. cit.*) it has been shown that whole molecules in a dissociated solution are poisonous and therefore have a physiological action, no application of the dissociation theory to chemotaxis can be considered complete until it has been determined what effect, if any, is due to the whole molecules.

**General Points.** If it is desired to determine whether a neutral salt attracts or not, experience teaches us that this may best be attempted by trial at a concentration of about  $\frac{1}{10}$  mol.

<sup>1</sup> E. g. Ostwald, *Grundriss der Allgemeinen Chemie*, 1<sup>86</sup> Aufl., 1889; also Whetham, *Solution and Electrolysis*, Cambridge, 1895, p. 165.

It was ingeniously proposed by Pfeffer<sup>1</sup> to use spermatozoa as a test for malic acid in the cell-sap of plants. He brought broken cells into a medium in which spermatozoa were swimming, and found that the latter were attracted by the diffusing cell-sap. He therefore concluded that malic acid was present in the cells. Since, however, it is now known that many other substances widely occurring in cell-sap such as tartrates, potassium oxalate, phosphates, &c., attract spermatozoa at fairly low concentrations ( $\frac{1}{100}$  mol), this test for malic acid in cell-sap cannot be considered decisive.

The cell-sap of a petiole of *Gunnera scabra*<sup>2</sup> contained, according to an analysis of de Vries: 0.56 gm % of substances containing the negative radicle of malic acid, 0.67 gm % potassium chloride, 0.01 gm % potassium phosphate, and 0.56 gm % glucose. Such a cell-sap would undoubtedly attract spermatozoa. The chief rôle in the attraction would be played by the malic acid compounds. The potassium chloride would, however, also attract strongly. The glucose would have no action whatever.

Another analysis of de Vries showed that the growing point of the stem of *Helianthus tuberosus* contained 0.91 gm % potassium nitrate. This is sufficient to cause a strong attraction, as is evident from Table I. From the cases of *Gunnera*, *Helianthus*, and others which cannot be quoted here, and from the fact that many substances determined to give a positive chemotactic stimulus, such as tartrates, oxalates, potassium salts, &c., occur very frequently in plant-cells, we may conclude that the attraction given by any cell-sap is not necessarily due to malic acid compounds, and may take place in their absence.

Pfeffer determined an attraction by the cell-sap of all the plants (about thirty) which he employed for his experiments. The plants included Algae, Fungi, Mosses, Ferns, Gymno-

<sup>1</sup> Pfeffer, loc. cit., Bd. i, p. 413.

<sup>2</sup> De Vries. Eine Methode zur Analyse der Turgorkraft, Jahrb. f. wiss. Bot., Bd. xiv, 1884, p. 574.



sperms, and Angiosperms. That the attraction was so general suggests that other substances beside malic acid compounds may have taken part in giving the results.

Not every solution of mixed salts, one or more of which latter attract, is capable of giving an appreciable attraction. Thus with a nutrient solution for higher plants I could not determine an attraction at any concentration. The salts were present in the following proportions by weight: calcium nitrate 100, potassium nitrate 25, sodium chloride 15, magnesium sulphate 50, and potassium phosphate ( $\text{KH}_2\text{PO}_4$ ) 25. An attraction might have been expected from the presence of the potassium salts. Its absence was probably due to the acid reaction of the phosphate. It is doubtful whether the non-attracting calcium nitrate and sodium chloride affected the result.

When the spermatozoa are liberated in distilled water I could determine no attraction by tap-water or by water from a small pond. These weak salt solutions are not sufficiently concentrated to give any chemotactic effect, although individual salts which they contain (when more concentrated) certainly attract. In these experiments the prothallia were cleaned as thoroughly as possible in distilled water before the spermatozoa were liberated.

Rubidium chloride was found to attract spermatozoa about as strongly as potassium chloride. Since other chlorides tested do not attract, the stimulus given by rubidium chloride appears to be due to the metal. Rubidium is a rare metal and only present in very minute quantities in the soil. When one reflects upon the kind of substratum upon which prothallia grow, it seems extremely improbable that rubidium salts ever gain access to the water-drops in which spermatozoa swim in sufficient quantity to cause attraction. We appear therefore to have, in my experiment with rubidium chloride, a case of a reaction to a stimulus without any special biological significance. Parallel cases seem to be given in the response of roots to the stimulus of light, electric currents and slow currents of water by alteration in the

direction of growth. Perhaps these useless reactions<sup>1</sup> are to be explained upon a theory of physiological correlation. The acquirement of one function may necessitate the acquirement of another. The apparatus required for the reception interpretation and reaction in the case of one stimulus may be of such a nature that it may be set in motion by another stimulus. Thus the apparatus acquired for performing biologically useful movements by response to malic acid may be such in the case of the spermatozoa of Ferns that it can also be employed by rubidium chloride to give directive movements which have no biological advantage.

## II. THE WITHDRAWAL OF WATER FROM SPERMATOOZOA.

When a certain amount of water is withdrawn from spermatozoa they come to rest entirely. If after having been brought to rest in this way the spermatozoa are again allowed to absorb water freely, resumption of movement may take place. Similar facts have been observed for Bacteria<sup>2</sup> and other organisms.

When a spermatozoon enters a capillary tube which contains a concentrated solution of a neutral salt, e.g. 5 gm % potassium nitrate, it moves slower and slower until, upon penetrating a certain distance into the tube, it ceases to move forward. The cilia, however, continue to move for some time, finally coming to rest. If the spermatozoon is carried out of the tube by the out-going under-current (see p. 556) the cilia recommence their motion. As less and less concentrated diffusion zones are reached the cilia move more and more rapidly. The spermatozoon soon begins to move slowly forward, the progression being in almost a straight line usually away from the mouth of the tube. Having arrived in the normal medium the organism moves more rapidly, but

<sup>1</sup> Useless reactions are also admitted by Goebel, Ueber Studium und Auffassung der Anpassungserscheinungen bei Pflanzen, Festrede, Akad. d. Wiss., München, 1898, p. 15.

<sup>2</sup> A. Fischer among others. Untersuchungen über Bacterien, Jahrb. f. wiss. Bot., 1895, p. 75.

still for a while in a fairly straight line. After a few minutes the spermatozoon moves once more at the normal rate and in the normal manner. The latter may be roughly described as a revolution in more or less complete circles about an ever-changing centre. I have noticed the above facts a considerable number of times in experiments with concentrated solutions. Recovery has taken place after a spermatozoon has been motionless for five minutes. Cessation of movement and death are therefore by no means necessarily simultaneous, as has been previously assumed<sup>1</sup>.

In order to determine the manner in which movement is affected by concentration the following method was used. Glass rings (10 mm high  $\times$  20 mm broad) were fastened upon microscope slides by means of a mixture of wax and fat, and fitted with cover-glasses. A little of the solution to be tested was poured into the chamber, and a hanging drop made. To the latter a single prothallium, after being well washed in part of the same solution, was added. The cover-glass was sealed with vaseline. By this method the spermatozoa were directly liberated into the solution to be tested. The solution could not alter appreciably in concentration, and oxygen had free access to it.

The solutions were made isotonic with 1,  $\frac{1}{2}$ ,  $\frac{1}{5}$ ,  $\frac{1}{10}$ ,  $\frac{1}{20}$  and  $\frac{1}{100}$  mol potassium nitrate. The solvent was tap-water.

In Table V, F indicates that the spermatozoa moved forward,

C „ „ only the cilia moved,

O „ „ there was no movement at all.

At the two highest concentrations of the salts and sugar spermatozoa were only rarely set free. The experiments were carried out with the spermatozoa of *Gymnogramme Martensii*. In the case of alcohol, potassium nitrate and cane-sugar similar results were obtained on repetition with the spermatozoa of *Pteris serratula*.

<sup>1</sup> Pfeffer, loc. cit., Bd. i, p. 385.



TABLE V.

Standard solutions of potassium nitrate			in parts of a mol in grams per cent.		1 10.1	$\frac{1}{2}$ 5.05	$\frac{1}{3}$ 2	$\frac{1}{10}$ 1.0	$\frac{1}{20}$ 0.5	$\frac{1}{100}$ 0.1
Potassium nitrate	KNO <sub>3</sub>	101	0	0	C	F	F	F	F	F
Sodium chloride	NaCl	58.5	0	0	C	F	F	F	F	F
Cane sugar	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	34 <sup>2</sup>	0	0	C	F	F	F	F	F
Glycerine	C <sub>3</sub> H <sub>5</sub> (OH) <sub>3</sub>	92	F	F	F	F	F	F	F	F
Alcohol	C <sub>2</sub> H <sub>6</sub> O	46	F	F	F	F	F	F	F	F

From the above Table it will be noticed that at the concentration  $\frac{1}{10}$  mol potassium nitrate, sodium chloride and cane-sugar the spermatozoa move forward. The movement is, however, not so rapid as at  $\frac{1}{20}$ . At the concentration  $\frac{1}{100}$  mol movement takes place in the same manner as in tap-water alone. At the concentration  $\frac{1}{3}$  mol the spermatozoa are still liberated, but after being set free from their mother-cells only move their cilia, and are not capable<sup>1</sup> of moving forward through the medium. The movement of the cilia often lasts a long time. I have watched such movement in 2.05 gm per cent. potassium nitrate for an hour. A certain number of spermatozoa liberated at the concentrations  $\frac{1}{10}$  and  $\frac{1}{3}$  mol never move at all. At the concentration  $\frac{1}{2}$  mol a few antheridia burst, but no motion of the spermatozoa was observed.

The spermatozoa were able to move forward in all the solutions of glycerine and alcohol tested. The difference in the behaviour of the organisms in solutions of these substances and in solutions of potassium nitrate, sodium chloride and sugar, is to be accounted for by the fact that the former two substances<sup>2</sup> rapidly penetrate living cell-membranes, whereas the latter three are practically excluded from penetrating at all. Solutions of alcohol and glycerine do not therefore cause the same physical disturbance, namely the

<sup>1</sup> Exceptionally a spermatozoon struggles a very short distance and very slowly through the medium.

<sup>2</sup> For literature, see p. 556.

withdrawal of water, as the salts and sugar. The movement in alcohol and glycerine at high concentrations is not surprising if we draw what seems to be the justifiable conclusion that the rate of motion of spermatozoa varies (within certain limits) as the amount of water they contain.

Glycerine penetrates living membranes much more slowly than alcohol. In my experiments it was found that at the concentration 1 mol of glycerine the spermatozoa did not immediately begin to move forward after liberation. After lying motionless for a short time they began to move slowly forward, at length reaching a maximum speed. In an isotonic solution of alcohol the spermatozoa moved quite rapidly as soon as liberated. The difference in the rate of penetration of the two substances into living membranes makes itself here clearly apparent. Glycerine requires a considerable time to penetrate. Hence the recovery of the spermatozoa. Alcohol penetrates almost instantaneously. Hence the rapid movement of the spermatozoa at the concentration 1 mol of this substance immediately after their liberation.

It was noticed that the spermatozoa did not move so rapidly in  $\frac{1}{10}$  mol potassium nitrate as in  $\frac{1}{10}$  mol cane-sugar. This result, which was repeated several times, may possibly be accounted for by the more toxic action of the salt. It may, however, indicate that the membranes of the organisms are to a slight degree penetrable by cane-sugar.

### III. LENGTH OF THE SWARM-PERIOD.

According to Strasburger<sup>1</sup> the swarm-period for the spermatozoa of *Pteris serratula* is not more than half an hour, while for *Ceratopteris thalictroides* it is scarcely twenty minutes. Voegler<sup>2</sup> has confirmed the latter observation and has also made the following additional swarm-period determinations:—*Gymnogramme Laucheana*, twenty-five minutes;

<sup>1</sup> Strasburger, Die Befruchtung bei den Farnkräutern. Jahrb. f. wiss. Bot., 1869-70, Bd. vii, p. 396.

<sup>2</sup> Voegler, loc. cit., Bot. Zeit., 1891, pp. 646-648.

*Dicksonia antarctica*, fifty to fifty-five minutes; *Blechnum occidentale*, thirty-five to forty minutes; species of *Alsophila*, *Polypodium*, *Asplenium*, *Neprolepis* and *Osmunda*, twenty-five to forty minutes. My own observations on *Gymnogramme Martensii* differ considerably from those made by Strasburger and Voegler for other spermatozoa. The swarm-period for this species is about 120 minutes.

In determining the length of the swarm-period, I have used the hanging-drop method already described (p. 573). A single prothallium with few antheridia was placed in a drop. It was thereby possible to watch the liberation of the spermatozoa. Several antheridia usually burst almost simultaneously. When it was thought that no more antheridia would burst the prothallium was allowed to remain in the drop. If the contrary was feared the prothallium was removed.

The time intervening between placing the prothallia in water and the bursting of the antheridia varies according to the ripeness of the latter. In a considerable number of experiments the time was ten to twenty minutes. After an antheridium has burst several minutes, often about eight, are required before the spermatozoa have all become set free from the mother-cells. In a few cases individual spermatozoa after liberation from an antheridium did not swarm at all. Sometimes a few spermatozoa were left in an antheridium after the others had escaped and there continued to move for a considerable time.

It was found that in tap-water the majority of spermatozoa move well for about two hours, after which they come to rest one by one, the longest time of movement being about three hours. This result is somewhat remarkable in view of the already quoted, much briefer swarm-periods given by Strasburger and Voegler for other spermatozoa. In particular, Voegler's estimation of the swarm-period for *Gymnogramme Laucheana* is only twenty-five minutes. According to my estimation the swarm-period for the nearly related *G. Martensii* must be considered at least five times as long.



The detrimental effect of distilled water upon the life of spermatozoa has already been remarked by Pfeffer<sup>1</sup>. In my experiments with hanging drops of distilled water it was found that the majority of spermatozoa came to rest in about thirty minutes, the longest time of movement being just one hour. The swarm-period was thus reduced to less than one-third of that obtained with tap-water. The distilled<sup>2</sup> water was not shaken up before use. I was under the impression that the shallow hanging drops exposed to the air in the glass-cells would almost immediately become saturated with oxygen. From the work of Senn<sup>3</sup>, with which I have since become acquainted, this appears to have been a mistake. Senn found that in hanging drops of distilled water which had not been shaken up with air *Coelastrum reticulatum* formed colonies or coenobia. In similar drops of distilled water which had been previously shaken up with air the Alga gave rise to single unconnected cells. The difference in the behaviour of the Alga in the two cases is due to the different amount of oxygen in the drops. Similarly the difference in the amount of oxygen in hanging drops of tap-water and of distilled water may determine the difference in the swarm-period of spermatozoa in the two cases.

Pfeffer<sup>4</sup> found that spermatozoa swarmed at least twice as long in an open drop of water as in an ordinary coverglass-preparation where the coverglass was supported by strips of paper, and at least five times as long as in an ordinary preparation not so supported. He attributed the differences in the length of the swarm-periods to the amount of oxygen present in the different cases. It is to be regretted that neither Strasburger nor Voegler have stated the exact methods which they employed in determining swarm-periods. If coverglass-preparations, which indeed Voegler<sup>5</sup> appears to have used, or hanging drops made with unshaken distilled water were

<sup>1</sup> Pfeffer, loc. cit., Bd. i, p. 368, Anm. 3.

<sup>2</sup> Doubly distilled and put in a specially cleaned two-litre flask.

<sup>3</sup> Senn, Ueber einige coloniebildende einzellige Algen. Dissertation, Basel, 1899, p. 13.

<sup>4</sup> Pfeffer, loc. cit., Bd. i, p. 372.

<sup>5</sup> Voegler, loc. cit., p. 646.

employed, much briefer swarm-periods would certainly result than would be the case if the spermatozoa were liberated in hanging drops of tap-water. With similar conditions it seems not improbable that there would be no great difference in the length of the swarm-periods of *Gymnogramme Laucheana* and *G. Martensii*.

Weak solutions of alcohol<sup>1</sup> made with tap-water were found to be less deleterious than the distilled water. Thus in 1.5 gm per cent. the spermatozoa move as long as in tap-water. In two experiments the times of longest movement were determined to be two hours fifty-eight minutes and three hours twenty-five minutes respectively. In each case the majority of spermatozoa moved well for about 100 minutes. With increasing concentration, however, the injurious effect of the alcohol becomes obvious. Thus in 6.9 gm per cent., although the spermatozoa move rapidly at first they come to rest in fifteen minutes. The fact, however, that the movements last so long in such concentrated solutions of alcohol proves that this substance is not so toxic as is often supposed.

In glycerine I have been unable to detect any toxic action. When spermatozoa are liberated in a hanging drop of 13.8 gm per cent. (the solution being made with tap-water) they are at first motionless, but after a few minutes begin to move slowly, gradually obtaining a maximum speed. It is probable that during this process the glycerine penetrates into the protoplasm. Some spermatozoa, however, do not recover and never move in these strong solutions. Although the motion is not so lively the swarm-period is quite as long in 13.8 or 6.9 gm per cent.<sup>2</sup> glycerine as in tap-water.

Acids are very toxic. Malic and tartaric acids are about equal in their effects. Thus at 0.01 gm per cent. the sper-

<sup>1</sup> In these experiments, as in all those made with the glass rings, the chamber was about half filled with the solution of which the hanging drop was made.

<sup>2</sup> In some experiments with this concentration movement still took place more than four hours after removal of the prothallia from the drops. Since some of the spermatozoa may have begun to move after lying some time motionless in the glycerine it cannot be assumed without further experiment that they swarm longer in a solution of glycerine than in tap-water.

matozoa do not move more than about ten minutes, and even at a concentration of 0.001 per cent. the swarm-period is reduced to about two-thirds the normal.

The swarm-period is also much reduced when the spermatozoa are liberated in solutions of neutral substances which are concentrated enough to withdraw a considerable quantity of water from the organisms. Thus at a concentration isotonic with 2 per cent. potassium nitrate there is no forward movement, no swarming, for either cane-sugar, potassium nitrate or sodium chloride. At concentrations isotonic with 1 per cent. potassium nitrate swarming takes place, but its period is much reduced. If, however, the hanging drops are isotonic with 0.1 per cent. potassium nitrate, the swarming is as active as in tap-water alone. The swarm-periods in these weak solutions were not exactly observed, but after forty minutes the spermatozoa were seen to move as actively as in similar experiments with tap-water.

#### IV. THE STARCH IN THE VESICLE.

It is well known that the spermatozoa of the Ferns carry with them a vesicle attached to the hinder end of their bodies. In this vesicle is usually a considerable quantity of starch in the form of small grains.

The starch can readily be distinguished in the living spermatozoa whenever they move slowly enough for the purpose. It appears to occur under normal conditions in all freshly liberated spermatozoa. If a prothallium be plunged in a solution of iodine, the antheridia become more darkly stained than any of the vegetative cells. This is due to the relatively large amount of starch stored up in the mother-cells of the spermatozoa. The starch grains, often about ten in number, are chiefly collected on the side of attachment of the vesicle.

According to Strasburger<sup>1</sup> the spermatozoa, on being attracted toward the archegonia, lose their vesicles in the

<sup>1</sup> Strasburger, *Die Befruchtung bei den Farnkräutern*, loc. cit., p. 403.



slime at the mouth. The vesicles are somewhat sticky, and sometimes become attached to various objects. A spermatozoid, in struggling to get free, may break away from its vesicle and lose it. Pfeffer<sup>1</sup>, however, has observed that, as a rule, the vesicle remains attached during the whole of the swarm-period. This is certainly the case with *Gymnogramme Martensii*.

Pfeffer<sup>2</sup> made some experiments to determine whether the starch disappeared during the swarm-period. He killed spermatozoa after they had been liberated for an hour, and compared the quantity of starch contained in the vesicles with that in the vesicles of spermatozoa when just set free. Although the quantity of starch appeared to have somewhat diminished during the swarm-period, he was obliged to admit, having repeated the experiment twice, that the result was not decisive.

It has already been stated that the spermatozoa of *Gymnogramme Martensii* move well for about two hours, after which they gradually come to rest, the time of longest movement being about three hours. I decided, therefore, to compare the amount of starch present in spermatozoa just liberated and such in spermatozoa after three hours' freedom. Similar prothallia from the same pot were used. The experiments were repeated three times. It was found that the starch had either greatly diminished in quantity or had disappeared entirely. A considerable number of spermatozoa, after being stained with iodine, were seen to be quite free from starch. On the contrary, all the freshly liberated spermatozoa contained the usual quantity, the large and small grains becoming very clear with the iodine reaction.

From the foregoing result, it would seem that Pfeffer's suggestion that the starch may be used as a food material during the swarm-period is justified, at any rate in the case of *Gymnogramme Martensii*. If, however, the swarm-periods for the spermatozoa of other species of Ferns are as short as those given by Strasburger and Voegler it is very doubtful,

<sup>1</sup> Pfeffer, loc. cit., Bd. i, p. 370.

<sup>2</sup> Ibid.

especially in view of the experiments of Pfeffer, if the starch has in general the significance here given it.

The vesicle swells considerably during the swarm-period, and must occasion considerable resistance in the motion of the spermatozoa through the water. If, however, the starch is used up as food this disadvantage might be more than compensated, and the arrangement of the spermatozoid and its vesicle be likened to a locomotive with its well-stored tender attached behind. It is, however, possible that the vesicle may be partly used to regulate the motion or as a balancing apparatus during the swarm-period. One can scarcely suppose that so comparatively large a structure is a useless vestige of the mother-cell.

#### V. SUMMARY OF THE CHIEF RESULTS.

In addition to malic acid and its salts many organic and inorganic salts widely occurring in cell-sap give the spermatozoa of Ferns a positive chemotactic stimulus.

Malic acid and its salts attract more strongly than any other substances tested.

It is not free malic acid, but may be one of its salts, which plays the chief rôle in attracting the spermatozoa to the archegonia.

Sugars, alcohols, asparagin, and urea do not attract.

The attracting neutral salts do not give an unmistakable tonotactic repulsion at high concentrations. If such a repulsion occurs it does not prevent the spermatozoa from finally entering concentrated solutions where they are brought to rest by loss of water.

The repulsion given by malic and maleic acids is chemotactic. The dissociation theory of solutions gives a clue for understanding the chemotaxis of the spermatozoa.

The attracting of the spermatozoa by cell-sap is not a decisive proof of the presence in it of malic acid compounds, and may take place in their absence.

The withdrawal of a certain quantity of water from the

spermatozoa brings them to rest. Recovery takes place on reabsorption of the water. The protoplasm of the spermatozoa is penetrated very slowly or not at all by sugar and neutral salts, rapidly by glycerine, and very rapidly by alcohol.

The swarm-period of the spermatozoa of *Gymnogramme Martensii* is about 120 minutes. Previously determined swarm-periods are much shorter.

The starch in the vesicles of the spermatozoa of *Gymnogramme Martensii* disappears during the swarm-period.

The work for the above paper was done between November, 1898, and August, 1899, during the term of an 1851 Exhibition Scholarship, in the laboratory of Professor Pfeffer at Leipzig. I have much pleasure in acknowledging with my best thanks his kind advice and stimulating criticism.



# The Development of the Archegonium and Fertilization in the Hemlock Spruce (*Tsuga canadensis*, Carr.).

BY

WILLIAM A. MURRILL<sup>1</sup>.



With Plates XXXI and XXXII.



THE material for these studies has been collected with great regularity for the past three years from a hemlock standing alone on the bank of a stream in an open pasture near the University grounds. The tree is well advanced in years and has fruited heavily every season. The female cones are terminal on the larger horizontal twigs, the male cones occurring in great abundance on the smaller ones. At pollination, there is no change in the position of the female cones; they remain slightly pendent, the scales opening a little and receiving the pollen from below as it floats upward. Soon after pollination, the pedicels lengthen and the cones, which are now considerably heavier, hang directly downward. Pollination on a single tree occupies about three days, but a week or more elapses before it is completed on all the trees of this locality. In 1899 it was at its maximum on May 19, in 1900 on May 22. The seasons frequently differ more than this.

<sup>1</sup> Read before the Botanical Society of America, at its sixth Annual Meeting in New York City, June 28, 1900.

[Annals of Botany, Vol. XIV. No. LVI. December, 1900.]

Two weeks after pollination the archegonial rudiments appear; a week later the necks are formed; and two weeks after this the ventral canal-cell is cut off. Fertilization takes place five days after the ventral canal-cell is formed. It varies for the same and different trees much as pollination does, so that stages of fertilization may be obtained for a week or longer. After one season's experience, it is possible to determine with tolerable accuracy the date of fertilization, but, after all, there is an element of chance that can be eliminated only by regular and abundant collections. I put up material from one to three times a day. The hour of collection seems to be of little consequence. The central cell was found in active division in cones collected at 10 a.m. in bright sunlight, in others collected at 7 p.m., and in still others on branches taken from the tree at 7 p.m., and kept in water until 11 p.m. Fertilization stages were abundant in cones collected at 9 a.m. and at 9 p.m. of the same day.

#### METHODS.

The material was placed in the fixing solution within a few minutes after it was taken from the tree. Only the middle portion of the cone was used, as this part contains the best-developed ovules. In the younger stages, the terminal sterile portion of the scale was cut away, leaving the two ovules attached side by side; in older stages, the ovules were entirely separated from the scale, and, as the coats became hardened, they were cut away at the sides, exposing the endosperm directly to the fixing fluid. The endosperm should be quite well filled out before this is done, otherwise it may collapse. After the embryos are well established, it is well to remove the coats entirely. Many approved fixing methods have been tried, with variations in strength, time, and temperature, but for these studies none has been found equal to Mottier's modification of Flemming's solution, used fresh and allowed to act for twenty-four hours at about 30° C. During the preparation and fixing of the ovules the bottles were repeatedly shaken to ensure equal contact of fresh

solution on all sides. The solution was sometimes changed at the end of one or two hours. Into each bottle was placed at the time of fixing a small rectangular piece of linen paper bearing in pencil the current number. This paper remained with the material through all of the succeeding changes, appearing finally at the bottom of the paraffin block as a permanent and very convenient label. After fixing, the material was washed for twelve to twenty-four hours in running water, dehydrated in grades of alcohol, bleached with a seventy per cent. alcoholic solution of hydrogen peroxide, and the dehydration completed in commercial and absolute alcohol several times changed. It was then brought very gradually into cedar oil, and transferred with equal care into paraffin melting at  $54^{\circ}$ , in which it was imbedded. I have found it expedient to store material in seventy or eighty per cent. alcohol for a short time after bleaching, and to allow a number of bottles to accumulate before proceeding farther with the imbedding. Time and chemicals are thus saved and more attention is given to the details of the process.

A Minot-Zimmermann revolving microtome was used in cutting the sections. In cases where the material was poor in stages, a number of ovules were imbedded in rectangular groups and sectioned together. Archegonia were thus examined by thousands instead of by hundreds. Sections were cut  $6.6\mu$  and  $13.3\mu$  in thickness. The ribbons were floated out on water, fixed to the slide with Mayer's albumen fixative, dried thoroughly, and melted down by placing the slides in the paraffin bath for an hour or two. The slides were numbered with figures and letters according to the system devised by Marks. A mixture of vermilion and sodium silicate in a little water supplied a convenient and indelible medium. Preliminary to staining, the slides were passed through xylol, alcohol, hydrogen peroxide solution and water. Several staining combinations were used, the well-known Safranin-Gentian-Violet-Orange G. combination proving the best; though Iron-Hematoxylin alone and combined with acid Fuchsin, and Delafield's Hematoxylin



alone and followed by Orange G. or Bismarck-Brown, were useful for comparison in some stages of archegonial development and oögenesis. Methyl-Green, Fuchsin, and Orange G. were used in the search for centrospheres and some slides were covered without staining. After the usual process of dehydration with absolute alcohol and clearing with oil of cloves, the sections were treated for several minutes with Bergamot oil before being covered with balsam. This removes foreign particles and ensures the permanence of certain stains that are extracted by the oil of cloves: it also flows easily and dries quickly.

#### THE ORIGIN AND DEVELOPMENT OF THE ARCHEGONIA BEFORE THE FORMATION OF THE VENTRAL CANAL-CELL.

The archegonia of *Tsuga* arise, as they do in other Gymnosperms, from superficial cells at the apex of the prothallium which cease to divide and become conspicuous for their large size and the abundance of protoplasm which they contain. Fig. 1, Plate XXXI, shows one of these archegonial rudiments with its large nucleus and its radiating bands of protoplasm, which, in a later stage (Fig. 2) are confined chiefly to the upper portion of the cell, while the lower tapering portion is comparatively empty. Between these rudiments are other cells which continue to divide (Fig. 3) and later give rise to the archegonial sheath (Fig. 4). About one week after the differentiation of the archegonial rudiment, the neck-cell is cut off (Figs. 2-3). It at first has the form of a circular disk surmounting the central cell and equalling it in breadth but not in height. As the central cell grows, it appears comparatively narrow and more elongated and also shows considerable variation. In the greater number of well-developed archegonia, it divides into two cells about the time when the ventral canal-cell is formed (Figs. 4-6). The division-wall may be transverse, oblique, or longitudinal, but it is most often oblique. In many cases the neck-cell remains undivided, and, on the other hand, one

frequently finds three or four cells in the neck of a mature archegonium (Figs. 8-10). Differences of opinion concerning the number of neck-cells in the archegonia of *Tsuga* are probably due to the fact that the division often occurs late. After examining a large number, I must agree with Hofmeister that two cells are more frequently present than any other number. Had Mottier (1892) examined more of the mature archegonia, he would have probably found two cells even more frequent than he supposed.

Returning to the condition of the central cell after the neck-cell was cut off, it will be remembered that it tapers towards its lower extremity and is almost entirely free from protoplasmic contents. Though its nucleus remains at the apex just beneath the neck, rapid changes take place in the form and contents of the cell. It increases greatly in size, and a delicate reticulum appears with numerous vacuoles in its meshes containing cell-sap (Fig. 11). Enveloping the central cell is a sheath of cells rich in protoplasm which furnish the central cell with food, the endosperm still growing vigorously and crowding back the disorganized nucellar tissue. In place of the delicate network shown in Fig. 11, the central cell soon shows spherical vacuoles filled with granules and other food-masses (Fig. 12). These appear first at the periphery near the food-cells and later come to occupy the whole cavity, with the exception of one or two large vacuoles at the centre. A transverse section of a prothallium with five archegonia at this stage is shown in Fig. 16.

I cannot confirm for *Tsuga* the results of Arnoldi's recent studies (1900) on the proteid vacuoles of the Abietineae. It may be that further search on my part will reveal the passage of the nuclei of the sheath-cells into the central cells, but very careful examination of numerous archegonia in all stages of development has thus far failed to show a single undoubted example of such passage. I find the nuclei of the sheath-cells staining diffusely at times, as described by Ikeno (1898) for *Cycas*, and I observe collections of granules in the outer vacuoles of the central cells which very much resemble

the sheath-nuclei, but the sheath-cells are never found without their nuclei. The sheath remains one-layered, though its cells often divide as the archegonium grows. At points where the archegonia come into close contact (Fig. 16), the sheath is frequently crushed and destroyed, but throughout most of its extent the cells and their nuclei continue active during the life of the archegonium.

#### THE FORMATION OF THE VENTRAL CANAL-CELL.

Shortly before the division of the central cell, an accumulation of cytoplasm may be observed beneath the nucleus a little to one side of the longitudinal axis of the archegonium (Fig. 13). This accumulation is a dense mass of fibres with small granules of uniform size scattered through it, the whole being continuous with the cytoplasm around it and taking the same brownish stain with the Flemming combination. From the first it is closely pressed against the nuclear membrane, and soon begins to push it inward in the form of blunt, unequal projections (Fig. 17). This fibrous mass continues to increase in size and to send out radiations far into the cytoplasm, thus forming a support for the free lower pole. These radiations grow at their free ends from the cyto-reticulum and extend in all directions, but are of necessity short on the side next the nucleus.

The spindle-fibres arise within this mass and grow upward against and press in the nuclear membrane, while they also draw to a point below and establish the lower pole of the spindle (Figs. 18–21). At the pole there is usually greater density and frequently a rounded granule (Fig. 24), but nothing could be discovered corresponding to a centrosome, even with the most favourable methods. Activity at the upper pole begins late and is always feeble. The cytoplasm between the nucleus and the neck-cell is all of a density intermediate between that of the ordinary cytoplasm and that collected at the lower pole. In a few cases a minute hyaline lenticular area was observed at the upper pole, resembling the collections of sap usually seen between the polar caps of



dividing vegetative nuclei and the nuclear membrane (Fig. 15). In later preparations this area seemed to have broadened and filled with delicate threads (Fig. 18) which later drew together above, but I cannot be sure of this<sup>1</sup>. Possibly the collection of sap is so small that it often disappears in fixing. Careful search was made for a similar collection at the lower pole, but without success.

The further development of the spindle is very rapid. The nuclear membrane disappears below and the spindle-fibres press into the nuclear cavity and connect with the linen network, already partially arranged in such a way as to continue the fibres to the chromosomes or through the centre of the cavity. The fibres soon become homogeneous below, while at their upper extremities they are still only rows of granules (Fig. 21). Nothing now remains in the nuclear cavity except the spindle-threads and the homogeneous chromatin segments. The large spreading bundle of fibres originating from below traverses about two-thirds of the nuclear cavity before connecting with those from the upper pole. The nucleus consequently becomes pear-shaped at this stage, with the upper end larger. As the spindle-fibres draw in towards the centre, the chromosomes are forced from their peripheral position and come to lie along the central part of the spindle (Fig. 23). At the same time the upper pole is somewhat elevated and appears as an abrupt but sharp point rising out of the nuclear cavity. Supporting fibres run from this pole to the cell-wall and in various directions through the neighbouring cytoplasm (Fig. 25).

In the plate-stage (Fig. 26), the chromosomes show the form and arrangement characteristic of that class of divisions recently denominated 'typical' by Strasburger (1900), and

<sup>1</sup> It is often impossible to demonstrate the existence of an extra-nuclear spindle-rudiment at the upper pole, but the fibres appear to collect in bundles within the membrane and to unite at one point when the polarity becomes more pronounced. The narrowing and consequent stretching of the spindle as the metaphase is approached would elevate the upper pole and give it the appearance of penetrating the membrane (Fig. 22). In *Larix Americana* there is still less cytoplasm above the nucleus than in *Tsuga*, which makes the difference in the two poles yet more marked.

are drawn to the poles as attenuated U's or V's (Fig. 27). The cell-plate is laid down from the centre outwards. It begins in the dispirem stage and keeps pace for some time with the growing daughter-nuclei (Figs. 29, 30). The fibres thicken at the centre, and the thickened portions fuse together at the side. The spindle then broadens out and stretches the young cell-plate until it connects with the wall of the mother-cell (Fig. 31). The original nuclear membrane does not entirely disappear until the daughter-nuclei are formed. It then splits up into slender fibres and fades away into the surrounding cytoplasm.

The division above described, while it resembles that described by Rosen (1895), Hof (1898), Nemec (1899), and others for the vegetative cells of various plants, in the extra-nuclear origin of the spindle and the details of its construction, nevertheless differs very strikingly from that type by reason of the fact that the spindle is at first asymmetrical and originates in a large fibrous mass beneath the nucleus, which appears early, grows to an immense size, and remains for some time after the division is completed, being enveloped during its existence by numerous fibres radiating from it far into the surrounding cytoplasm.

Various authors (Ikeno, 1898, p. 567; Blackman, 1898; Chamberlain, 1899, figs. 1, 2; and others) have described or figured the nucleus of the central cell as remaining at the cell-apex until division occurs, and it would be interesting to know whether more careful study would show such a method of spindle-formation as I have discovered in *Tsuga* in these or similar cases of unequal division.

This collection of cytoplasm is probably due to the need of a support for the free lower pole, and the numerous radiations from it doubtless add greatly to its stability. The origin and growth of the spindle chiefly on the lower side of the nucleus seem to indicate that the force controlling division is largely centred there.

FURTHER CHANGES IN THE EGG AND THE VENTRAL  
CANAL-CELL BEFORE THE ENTRANCE OF THE  
SPERM-CELLS.

When the nuclear membrane is deposited about the young egg-nucleus, the chromatin is in the form of a thick homogeneous band which is gradually drawn out until the chromatin granules appear distinct on the linin threads. At the same time the threads anastomose to form an ordinary cell-reticulum, in which several small nucleoli appear (Figs. 29-31). The nucleus increases rapidly in size and begins to move down toward the centre of the egg. The chromatin now occupies chiefly the upper half, the lower part being comparatively empty (Fig. 32). As it begins to move, it usually becomes elliptical in form (Fig. 33), and remains so until fertilized, but if the archegonium is very broad it may remain perfectly spherical. As it increases in size, it takes in considerable food-material and the network becomes coarser and the nucleoli larger. When the centre of the egg is reached, it remains stationary in the resting condition until fertilized. The granules in the cytoplasm of the egg are arranged in rows radiating from the nuclear membrane. This arrangement appears with the formation of the egg and disappears with fertilization.

The nucleoli of the egg-nucleus first appear as minute spheres hardly distinguishable from collections of chromatin. They increase rapidly in size, however, and apparently unite to form larger ones and often a single one of immense size (Fig. 35). When first formed, these larger ones are very plastic and easily take the sickle-form under the influence of fixing agents. Later, the outer shell becomes firm and dense and takes a deep purple stain with Gentian-Violet, while the vesicular mass within stains feebly with Orange, and shows a delicate reticulum probably consisting of dissolved proteid substance precipitated by chromic acid. The shell is frequently found broken in mounted preparations, and the nucleolus then



suggests a sperm-nucleus with its membrane breaking away, and the contents dissolving in the nuclear substance of the egg.

The large vacuole at the centre of the egg changes its position as the nucleus moves down, and, passing it on one side (Fig. 34), comes to lie near the apex of the cell. The vacuole is apparently somewhat diminished in size as the egg-nucleus increases, and it is not improbable that some of the cell-sap from the vacuole passes into the nucleus while they lie near together. The concentration of cytoplasm and food materials near the centre, in consequence of the change in position of the nucleus, leaves more room for the vacuole near the apical portion of the egg. Another vacuole with contents very similar to those of the nucleus is regularly observed<sup>1</sup> in the mature egg situated just beneath the ventral canal-cell (Fig. 10). It is quite different from the proteid-vacuoles in appearance, but seems to originate in one or more of the latter upon the relief of pressure in the apical region by the enlargement of their enclosing membranes and the expansion and distribution of their contents. This 'nuclear' vacuole sometimes fuses with the 'empty' vacuole as it approaches the apex, but the latter more often remains to one side and a little below the former. Near these two vacuoles is the receptive spot of the egg.

The ventral canal-cell is fairly persistent in *Tsuga*. When division is completed its nucleus is equal in size and similar in structure to the nucleus of the egg, and for some time shows the same stages of development. But when the nucleus has nearly filled the ventral canal-cell its membrane becomes wavy in outline, the scanty reticulum occupies only a small portion of the nuclear cavity, and the nucleoli remain minute and scattered. Growth having ceased, signs of disorganization soon appear. The nuclear contents become amorphous and stain diffusely, while the nucleus and the cell become more

<sup>1</sup> An archegonium rendered very abnormal by developing far down on the side of the prothallium showed this nuclear vacuole in all respects normal.

irregular and misshapen in outline. It is at this stage that the pollen-tube usually enters the neck of the archegonium.

THE ENTRANCE OF THE CONTENTS OF THE POLLEN-TUBE INTO THE EGG.

The pollen-tube reaches the egg by penetrating the neck of the archegonium, the contents of the neck-cells being pressed to one side or swept away entirely. They are frequently found crowded down near the apex of the egg with the remains of the ventral canal-cell (Fig. 36). On reaching the egg the tube spreads out over it and causes its apex to stain diffusely. Further than this the tube itself does not go, but the contents of its terminal portion are emptied into the egg near the empty vacuole, the membrane of the egg usually closing up again, but sometimes remaining open at the point of entrance.

The contents of the pollen-tube thus cast into the egg consist of two sperm-cells, the stalk-cell, the vegetative nucleus, and some protoplasm and starch from the tube-cavity (Fig. 36). The stalk-cell nucleus is small, perfectly spherical, and conspicuous by reason of its thick, deeply-stained reticulum (Fig. 39). The cytoplasm accompanying it is scanty, vacuolate, and irregular in outline. The vegetative nucleus is larger, ovoid or irregular in outline, and unaccompanied by cytoplasm. It contains a delicate network with granules and nucleoli, and takes a pale, slightly diffuse stain with Gentian-Violet.

Above these smaller nuclei lie the ellipsoidal sperm-cells, each with dense cytoplasmic contents and a large nucleus. The sperm-nuclei differ considerably in size and appearance. The one that was in advance in the pollen-tube, which I shall call the first sperm-nucleus, is about twice the diameter of its companion, and its contents are so dense that its two small purple-staining nucleoli are almost hidden from view. The second sperm-nucleus is less dense and shows two large and prominent nucleoli that stain a clear red with safranin. It tends to conform more to the shape of its cell, and also corresponds more nearly to the resting stage than does the larger nucleus.

It is through the first sperm-nucleus that fertilization is accomplished. A short time after its entrance into the egg it slips from its cell and moves with accelerated velocity towards the egg-nucleus, the latter remaining stationary and inactive, but probably exercising chemotactic attraction on the sperm-nucleus by reason of its rich proteid contents. There is no depression at the apex of the egg-nucleus, nor other evidence in it of the approach of the sperm. The conjugation-path is a straight line from near the point of entrance. Since this point is ordinarily at the side near the top of the egg, the sperm-nucleus usually strikes the egg-nucleus slightly to one side of its apex; when entrance into the egg is effected at the apex, the sperm-nucleus strikes the egg-nucleus directly at its apex (Fig. 38). Conjugation occasionally occurs at the middle of one side of the egg-nucleus, as shown in Fig. 40. The same figure also shows the second sperm-nucleus very near the egg-nucleus, but there is nothing in this or other preparations of mine to indicate that both sperm-nuclei ever unite with the nucleus of the egg.

In Conifers, the sperm-cell is very similar to a pure vegetative cell. In the Pteridophytes, the nucleus is condensed and elongated, with reduced cytoplasm and active cilia. In Phanerogams, the nuclei may be elongated, but cytoplasm and cilia are absent. The sperm-cells of *Ginkgo* and the Cycads differ from those of the Conifers in possessing a cilia-bearing band which propels the cells from the tip of the pollen-tube to the apex of the egg. In the Conifers, the pollen-tube penetrates to the egg and the cilia-bearing band is unnecessary and absent.

After the first sperm-nucleus has moved to the egg-nucleus the second sperm-nucleus remains for a long time in its cytoplasm in the upper part of the egg (Fig. 38), and is then gradually absorbed, usually after the other contents of the pollen-tube have disappeared. The discovery of a tripolar spindle (Fig. 46) in the position commonly occupied by the second sperm-nucleus at first led me to believe that, being



fed by the egg-cytoplasm, this nucleus had entered upon mitotic division, just as the functional sperm-nucleus presumably initiates the division which, with the assistance of the egg-nucleus, results in the first segmentation. In the nuclei of unfertilized eggs, also, the process of disintegration may be accompanied by the fusion of the chromatic reticulum into rods similar to chromosomes, while the fibres of the nuclear membrane focus on several points external to the membrane as the nuclear cavity diminishes, thus forming a figure resembling a multipolar spindle. Occasionally, before the nucleus begins to diminish in size, the disintegrating chromatin contents are found collected near together, with numerous radiations present in the nuclear cavity, the whole suggesting a possible stage of fertilization.

#### FERTILIZATION AND THE FIRST SEGMENTATION.

When the egg-nucleus is reached, the sperm-nucleus flattens itself against it in the form of a bi-convex lens and soon comes to lie within its original boundary (Fig. 37). The surface of separation is at this time quite even and is composed of the two nuclear membranes with some included cytoplasm. The reticulum in the apex of the egg-nucleus is pressed down in advance of the sperm-nucleus and furnishes the first deposit of chromatin at that point.

The difference in the density of the sperm-nucleus and egg-nucleus is very apparent when they are thus first in contact, but the former begins almost immediately to lose its density and to become a perfect resting nucleus like that of the egg. In the process, no stainable substance is cast out into the cytoplasm, such as is described by Ikeno (1898) for *Cycas*, and Wager (1899) for some *Phycomycetes*; nor can any of its contents be seen to pass through the membranes into the cavity of the egg-nucleus; but increase in the number or in the size of the nucleoli of the sperm-nucleus is very evident (Figs. 41, 42). The deeply staining nucleoli occupy the centre of the nucleus, while its periphery shows a delicate chromatic

reticulum, which may have been present before, concealed by the dense contents of the nucleus, or may arise only after the contact of the sexual nuclei. Certain granules of this reticulum now gradually become larger by the addition of neighbouring granules, and the whole contracts to a coarse, knotted, slightly anastomosing thread, which, with the assistance of the nucleoli, passes over into the spirem band (Figs. 43-45).

While the sperm-nucleus has thus been entering upon the early stages of division, the chromatin of the egg-nucleus has been collecting near the centre of the nuclear cavity not far from the membranes separating the two nuclei. It likewise presents the appearance of an anastomosing, knotted thread, in contact with granules and spheres of various sizes apparently derived from the nucleoli, which latter now become hollow and stain feebly, and finally disappear.

Changes have also occurred meanwhile where the nuclear membranes are in contact. Instead of the even surface presented at the first contact of the nuclei, the membranes are now separated by numerous spherical granular areas, which tend to encroach on the cavity of the egg-nucleus and cause its membrane to show in cross-section a series of crenate folds. The contents of these spheres stain very slightly, with the exception of one or two small spherical bodies which are precisely like nucleoli and take the nucleolar stains. The disappearance of the membranes and the consequent union of the two nuclear cavities first occurs at points between these granular spheres, and the latter continue to occupy their position until the appearance of the spindle-fibres among them, when all of them disappear except a few upon which the fibres are centred. Whether they have any direct connexion with the formation of the spindle, or are simply cavities between the nuclear membranes containing a small amount of cytoplasm caught between the conjugating nuclei, it is impossible for me to say. Their increase in size with the decrease in density of the sperm-nucleus has suggested to me the arrangement found in *Cycas* and *Cephalotaxus*, where fusion is accelerated by root-like projections of the sperm-

nucleus into the egg-nucleus; but, even if the analogy were otherwise perfect, the increase in the amount of nucleolar substance seems to fully account for all the contents of the sperm-nucleus not found in its chromatic reticulum.

The breaking down of the membranes separating the two nuclear cavities occurs before the completion of the spirem bands, but the chromatin masses remain distinct until the chromatic segments appear. The achromatic contents of the cavity, however, undergo a decided change, becoming denser and more fibrous in appearance with the probable rearrangement of the linin network under the stimulus causing division (Fig. 45). The outer membrane also disappears in places, and some of the cytoplasm presses into the cavity, but the chief activities of division are intranuclear. With the union of the nuclear cavities there also occurs a change in the cytoplasm of the egg. The dense sheath of small granules and fibres that encircles the unfertilized egg-nucleus partly disappears, and the larger cytoplasmic granules extend almost to the nuclear cavity, while the rows of elongated granules, radiating into the surrounding cytoplasm from the egg-nucleus since its origin, now lose their radial position and are distributed without special arrangement. The indications are that the egg-nucleus has relaxed its hold for a time upon its cytoplasm to enter upon the changes involved in division.

The fibres originating the spindle of the first segmentation arise among the segmenting spirems derived from the two nuclei, and they first draw together at several different points forming a multipolar spindle-rudiment. On these fibres the long bent and twisted chromosomes appear, still showing the chromatin disks distinct on the linin thread (Fig. 47). As the number of fibres increase, and the spindle becomes monaxial in form, the chromosomes contract and become homogeneous, and are mostly bent in the form of a U. There is no difference to be observed between the chromosomes of the sperm and those of the egg, and, at this stage, they are mingled indiscriminately near the centre of the common spindle.



The mature spindle is broad, with many fibres and rather blunt ends, and, during the metaphase, the twenty-four chromosomes occupy all of the equatorial plane (Figs. 50, 51). The type of this division, so far as the chromatin is concerned, is the same as that already described for the central cell of the archegonium. The position of the mature spindle in the nuclear cavity seems independent of the relative position of the conjugating nuclei, and, while the division is usually oblique, it may be perpendicular to, or parallel with, the longitudinal axis of the archegonium (Figs. 52, 54, 57).

The chromosomes pass to the poles as slender undulated V's or U's, and fuse to form the network of the daughter-nuclei (Figs. 53, 54). No cell-plate is formed, but the fibres fade away into the cytoplasm, the slightly thickened middle portions being the last to disappear.

#### THE PRO-EMBRYO.

The two free nuclei resulting from the first segmentation increase rapidly in diameter, and soon divide simultaneously without change of position to form four nuclei of equal size lying free near the centre of the egg (Figs. 54-58). The type of the second division is similar to that of the first, but the spindle is narrower and more pointed at the ends (Fig. 56). The daughter-nuclei are formed and the spindle-fibres absorbed as in the first division. When the four nuclei have attained their full size, they move to the base of the archegonium (Figs. 59, 60), and by successive divisions in a horizontal plane give rise to four tiers of nuclei with four nuclei in each tier, as has been already described for various Conifers.

When the four free nuclei are moving through the egg-cytoplasm they do not show any special collections of fibres about their membranes, but soon after the base is reached they become enclosed in a dense mass of fibrous substance (Fig. 60), which supplies the material for the walls that appear later. Above this fibrous mass is a zone showing a delicate reticulum almost devoid of stainable substance. Above this

zone the egg shows its normal structure. The cause of this hyaline zone is difficult to determine. It appears that the stored food has been taken from this region in order to provide for the growing want of supplies for the nuclei below; but since the remaining available contents of the egg are doubtless also transferred to the developing embryo, there is no apparent reason why this zone should be left. It possibly represents an intermediate condition of these contents in which they do not easily take or retain stains.

#### SUMMARY.

The archegonia of *Tsuga* originate as single superficial cells, in each of which occurs the usual division cutting off an outer smaller cell that forms the neck. At maturity, the neck most commonly consists of two cells, though in very many cases the neck-cell fails to divide at all. Three and four cells in the neck are less commonly observed.

In the division of the central cell the spindle-fibres arise from a large dense fibrous mass beneath the nucleus and grow into the nuclear cavity, where they are later joined by fibres from the very small upper pole. The division resembles that recently described for many vegetative cells, but belongs to a new and distinct type hitherto undescribed. The mass of cytoplasm at the lower pole with its extensive radiations suggests a huge centrosphere.

As the egg-nucleus increases in size and moves to the centre of the egg, the vacuole moves upward, passing it on its way, and comes to lie near the egg-apex. Just beneath the ventral canal-cell may always be found another somewhat smaller vacuole with contents similar to those of the nucleus. These vacuoles form the receptive spot of the egg.

The contents of the pollen-tube cast into the egg consist of the vegetative nucleus with some cytoplasm and starch-grains, the stalk-nucleus surrounded by its own scanty, vacuolate cytoplasm, and the two unequal sperm-cells with their dense cytoplasm and large deeply-stained nuclei. The larger

sperm-nucleus slips from the cell and conjugates with the egg-nucleus, the smaller one being gradually absorbed with the other structures derived from the pollen-tube.

The functional sperm-nucleus flattens itself against the egg-nucleus in the form of a bi-convex lens, and the two nuclei soon come to lie in the space formerly occupied by the egg-nucleus alone, their membranes, however, remaining intact for a long time. In this condition the sperm-nucleus rapidly loses its density and constructs a delicate peripheral chromatic reticulum and larger central nucleoli, thus becoming a perfect resting nucleus similar to that of the egg. The chromatin of each nucleus collects in the form of a thick knotted thread near the centre of the separating partition, and the two masses remain distinct until the spirem-bands begin to segment.

Just before the spirems are formed the separating membranes disappear and the nuclear cavities become united. The spindle then arises in a multipolar fashion between and among the two masses, twelve chromosomes being supplied from the chromatin of the sperm and twelve from that of the egg, as described by Blackman for *Pinus Sylvestris*. The mature spindle is broad with blunt ends, and the manner of division is typical.

A second division succeeds the first without much delay, and the four resulting free nuclei soon attain full size and move to the base of the archegonium, where the young embryo becomes established in the manner already so well known among Conifers.

The investigations leading to the results recorded above were conducted in the Botanical Laboratory of Cornell University under the direction of Professor George F. Atkinson, at whose suggestion this work was undertaken, and for whose kindly sympathy and invaluable aid in its prosecution I am deeply grateful.



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## EXPLANATION OF FIGURES IN PLATES XXXI AND XXXII.

Illustrating Dr. Murrill's paper on *Tsuga Canadensis*.

Most of the figures were first drawn with the aid of a camera lucida from a Zeiss microscope, using compensation ocular 12 and the 2<sup>mm</sup> homogeneous objective, and then mechanically reduced to the present scale. For some it was necessary to use a lower magnification in the first instance. In using the camera lucida, I found it convenient to employ dark shades of paper (Andrews, 1898), usually black or dark blue, and the outlines were traced upon it in Chinese white with a long pointed pen. Since most of the preparations are coloured with dark stains, a white medium is peculiarly suitable for outlining chromatic structures.

### PLATE XXXI.

Fig. 1. ( $\times 400$ .) A portion of the apex of the prothallium, showing an archegonial rudiment in the centre, with rudimentary sheath-cells on each side.

Fig. 2. ( $\times 400$ .) The archegonial rudiment has increased in length, and its nucleus is preparing to divide. Almost all the protoplasm is collected at the upper end of the cell. The sheath-cells have increased in number.

Fig. 3. ( $\times 400$ .) The archegonial rudiment has divided into an inner central cell and an outer neck-cell. The outer cell is much smaller than the inner.

Fig. 4. ( $\times 200$ .) The neck-cell is in division. The central cell is very much larger, and is being rapidly filled with protoplasm from the rich layer of sheath-cells that closely envelop it. Its nucleus remains near the neck-cell and is still in the resting stage, though the condensation of cytoplasm just below it indicates that division is not far off.

Fig. 5. ( $\times 400$ .) A later stage of the same, showing the anaphase of the division in the neck-cell.

Fig. 6. ( $\times 400$ .) A still later stage, showing the neck-cell divided into two cells. The central cell is now entering upon division.

Fig. 7. ( $\times 200$ .) A mature archegonium, with two cells in the neck. The division wall is oblique. The ventral canal-cell is also shown.

Fig. 8. ( $\times 200$ .) A mature archegonium, with three cells in the neck. The first division was transverse, and the upper cell afterwards divided longitudinally.

Fig. 9. ( $\times 200$ .) Four neck-cells are present, formed by one longitudinal division followed by two oblique ones. The cytoplasm of both cells has divided, but no separating walls could be observed.

Fig. 10. ( $\times 200$ .) In this archegonium the four neck-cells are in one row, formed by two successive transverse divisions. Beneath the neck is the disorganizing ventral canal-cell, and beneath the ventral canal-cell, at the apex of the egg, is the nuclear vacuole. The empty vacuole is in another section. At the centre of the egg is the large resting nucleus. About the periphery of the egg, proteid vacuoles are abundant. The larger granules of cytoplasm are arranged in rows radiating from the nucleus. The contents of the egg have become so dense that its reticulum is wellnigh concealed. In many places, also, the reticulum has been distorted by denser aggregations of nutritive materials.

Fig. 11. ( $\times 200$ .) This follows Fig. 3 in the order of development, but shows a marked increase in the size of the central cell, with a well-formed sheath. The nucleus is at the apex of the cell, where it remains until division. Throughout the cell-cavity a delicate reticulum has been constructed, which is interrupted at the centre of the cell by a large vacuole filled with sap.

Fig. 12. ( $\times 200$ .) A later stage in the development of the archegonium. The neck-cell has elongated. The central cell has enlarged, and the meshes of its reticulum are fast filling with granular food supplies. The first proteid-vacuoles have been formed.

Fig. 13. ( $\times 400$ .) This figure represents the nucleus of the central cell preparing to divide. Its reticulum has become coarser and stains more deeply, and is balled up in the centre of the nuclear cavity in a condition suggesting synapsis. The preparations showing this condition are fixed as perfectly as one could desire. Beneath the nucleus is a dense fibrous mass closely pressed against the nuclear membrane, and sending out radiating fibres into the cytoplasm.

Fig. 14. ( $\times 800$ .) The spindle-fibres are arising in this mass, and growing upward against the membrane.

Fig. 15. ( $\times 800$ .) A lenticular hyaline area at the upper pole, seen in only two or three preparations, but very distinct and apparently perfectly normal.

Fig. 16. ( $\times 150$ .) A cross-section of a prothallium containing five archegonia of the stage shown in Fig. 12. It will be observed that each archegonium is enveloped by its own one-layered sheath. The archegonia are, therefore, ordinarily separated by two rows of sheath-cells, but at points where there is some distance between the archegonia the cells of the two sheaths have divided to fill the space. Between the two upper archegonia in the figure, the sheaths have been crushed to a line. This is quite frequently the case where the curved surfaces of the archegonia come nearest together.

Fig. 17. ( $\times 800$ .) Following Fig. 14, and showing the pressing in of the nuclear membrane, in the form of blunt protuberances, by the spindle-fibres originating below. The chromatin thread is now peripheral and almost continuous, though the disks are still far apart.

Fig. 18. ( $\times 800$ .) The spindle-fibres have advanced still farther, and preparations are being made within the nuclear cavity for their continuation. Segmentation occurs about this time, and rows of granules and delicate threads connect the chromatic segments with the incoming spindle-fibres. The greater



part of the chromatin is in the upper half of the nucleus. Activity has now begun at the upper pole, where delicate fibres are seen growing down against the nuclear membrane, but it rarely appears so distinct as in this preparation.

Fig. 19. ( $\times 800$ .) This nucleus belongs to a larger archegonium than that shown in Fig. 18. The spindle is in about the same stage, but the chromosomes are more advanced than those shown in the preceding figure. Radiations are present at the upper pole, and possibly a small polar cap, but the latter, if present, is not distinct enough to figure.

Fig. 20. ( $\times 800$ .) This figure represents the same stage as Fig. 19, but the nucleus remains spherical, and the spindle-fibres seem to have originated at a greater distance than usual below the nuclear membrane, and to have advanced with a more even front. This is exceptional, being observed only a few times in the examination of a large number of preparations.

Fig. 21. ( $\times 800$ .) The nuclear membrane has disappeared opposite the poles, and the spreading cone-shaped bundles of spindle-fibres have grown into the nuclear cavity, and are uniting somewhat above the centre of the nucleus. The true pear-shaped form of the nucleus and the inequality of the two poles cannot be shown in a longitudinal section which includes the upper pole, since the division is oblique and much of the lower pole is cut away. When first formed, the spindle is broad and the chromosomes, which have now become homogeneous, are attached to its outer threads.

Fig. 22. ( $\times 800$ .) The spindle-fibres are now homogeneous throughout, and the spindle has narrowed and drawn in the completed chromosomes nearer to the centre. In narrowing, it has also elongated, and the upper pole has been elevated. The section is made as in Fig. 21, and does not show all of the lower pole.

Fig. 23. ( $\times 800$ .) Further narrowing of the spindle has taken place, and the chromosomes are now coming up to the equator, preparing to enter the plate stage. The equatorial portion of the nucleus approaches very near the wall of the egg, and above this line the small cap of cytoplasm is dense and full of fibres.

Fig. 24. ( $\times 400$ .) The lower pole of the mature spindle is here represented. The fibres come to a definite point, and, in this case, focus on a small hyaline granule, which shows none of the properties of a centrosome.

Fig. 25. ( $\times 400$ .) The fibres of the mature spindle also converge to a definite, though rather abrupt, point at the upper pole, from which supporting fibres extend to the cell-wall. The remainder of the nuclear membrane also appears to function as a support to the spindle during metakinesis.

Fig. 26. ( $\times 800$ .) Separation of the chromosomes has begun at the nuclear plate, the bundles of mantle-fibres being attached on opposite sides of the diamond-shaped openings in the chromosomes already seen in earlier stages.

Figs. 27, 28. ( $\times 600$ .) The daughter-chromosomes pass to the poles as U's or V's with undulated margins. After they reach the poles, the central spindle-fibres appear lax, and the spindle becomes slightly concave in the equatorial region. This may be due to artificial causes or to relaxation after removal of the strain.

Fig. 29. ( $\times 400$ .) The daughter-chromosomes have united into a close, deeply-staining spirem, but no membrane is yet formed about them. The inner spindle-fibres are beginning to thicken in the equatorial region preparatory to the formation of the cell-plate.

Fig. 30. ( $\times 400$ .) The dispirems have opened out, but their loops still maintain a position parallel with the axis of the spindle. Delicate nuclear mem-

branes have been deposited about the daughter-nuclei, while the last traces of the membrane of the mother-nucleus have disappeared. The large dense mass of cytoplasm at the lower pole, however, still remains, and is present even when the egg-nucleus begins to pass down. The formation of the cell-plate is proceeding *pari passu* with the increase in diameter of the daughter-nuclei.

Fig. 31. ( $\times 400$ .) The daughter-nuclei have reached the resting stage, and show a delicate reticulum with several nucleoli. The egg-nucleus is somewhat larger than that of the ventral canal-cell. The cell-plate has been continued to the wall of the mother-cell.

Fig. 32. ( $\times 400$ .) The egg-nucleus is now not only larger than the ventral canal-cell nucleus, but its reticulum is growing more rapidly. The chromatic contents of the nucleus are mostly confined to its upper portion, the lower part containing chiefly nuclear sap.

Fig. 33. ( $\times 400$ .) The reticulum of the egg-nucleus has become coarser and the nucleoli larger. The nucleus has also changed its form from spherical to ellipsoidal, and has begun to move through the dense polar cytoplasm toward the centre of the egg. The larger granules of the general cytoplasm are arranged in radial rows about the nucleus, and the granules themselves are radially elongated.

Fig. 34. ( $\times 250$ .) At this stage the reticulum of the egg nucleus is distributed throughout the entire nuclear cavity. As the archegonium is unusually broad, the nucleus has retained its spherical form. Passing it on the left is the large central vacuole which now takes a position near the egg apex. The nucleus of the ventral canal cell is irregular in outline, and its contents show signs of disorganization. Traces of the division-spindle still remain above the nucleus.

#### PLATE XXXII.

Fig. 35. ( $\times 300$ .) The nucleus here represented is drawn from an egg into which the contents of the pollen-tube have just been discharged. Situated near the base of the nucleus, is the very large nucleolus with its firm, deeply staining outer shell broken at one point. The contents of the nucleolus appear finely granular and vacuolate in stained preparations.

Fig. 36. ( $\times 200$ .) The contents of the pollen-tube have entered the egg near its apex on the right hand side in the figure, and now lie beneath the empty vacuole with the functional sperm-nucleus in advance, and already free from its cytoplasm. Near it on the left is the stalk-cell, while between it and the sperm-cell lies the vegetative nucleus. The contents of the first sperm-nucleus are very dense and stain deeply. The egg-nucleus is apparently unaffected by the near approach of the sperm. Just above the egg are the remains of the neck-cells and the ventral canal-cell pushed aside by the entering pollen-tube.

Fig. 37. ( $\times 200$ .) The first sperm-nucleus has flattened itself against the apex of the egg-nucleus in the form of a biconvex lens. Its contents have as yet undergone no change. The second sperm-nucleus remains in its cytoplasm above, showing its reticulum and nucleoli very distinctly. The smaller nuclei are in another section. The first sperm-nucleus has left slight traces of its passage to the egg-nucleus in the intervening cytoplasm. These traces are more distinct in Fig. 38.

Fig. 38. ( $\times 200$ .) The sperm-nucleus has lost its density, and is now a true

resting nucleus like that of the egg. The membranes of the two nuclei are still intact. The changes in the two nuclei may be better described under later figures. In this case the stalk-cell lies near the second sperm-nucleus.

Fig. 39. ( $\times 1600$ .) The stalk-cell enlarged from the section represented in Fig. 38 to show its thick nuclear reticulum, and scanty vacuolate cytoplasm.

Fig. 40. ( $\times 200$ .) This figure shows the first sperm-nucleus in contact with the egg-nucleus, and the second sperm-nucleus almost touching its membrane. The sperm-cells entered the egg unusually far down on its side, and the bursting of the pollen-tube was sufficient to force them to this position near the egg-nucleus. This also accounts for the contact of the first sperm-nucleus at the side instead of the apex. The remains of the neck-cells could not be found. The egg-membrane did not close up again after the contents of the pollen-tube entered, as was the case in the egg represented in Fig. 36.

Fig. 41. ( $\times 400$ .) This represents a stage succeeding that shown in Fig. 37. The contents of the sperm-nucleus are losing their density, and numerous small spheres have appeared in the nuclear cavity. Crenate folds are observed in the membrane of the egg-nucleus at the surface of contact of the two nuclei.

Fig. 42. ( $\times 400$ .) The dense contents of the sperm-nucleus have disappeared except at the centre, and the nucleoli are larger and fewer in number. About the periphery of the sperm-nucleus a chromatic reticulum is seen. The chromatic contents of the egg-nucleus have begun to migrate to a point beneath the sperm-nucleus.

Fig. 43. ( $\times 400$ .) The centre of the sperm-nucleus is quite free from chromatic contents. The peripheral reticulum shows larger collections of chromatin. Nearly all of the chromatin of the egg-nucleus has collected beneath the sperm-nucleus. The crenate folds in the nuclear membrane of the egg appear larger, particularly in the centre.

Fig. 44. ( $\times 400$ .) The chromatin of the sperm seems to have moved nearer to that of the egg. The intervening membranes are preparing to break up. The egg-nucleus has constructed a chromatic reticulum.

Fig. 45. ( $\times 400$ .) The membranes have broken up, and the two nuclear cavities are continuous. Small spheres with granular contents now occupy the position formerly occupied by the membranes. The thick knotted chromatic threads of the two nuclei still remain distinct. All traces of nucleoli have disappeared, and the contents of the nuclear cavity have become denser and more fibrous. At a few points, the outer nuclear membrane is disappearing, and the cytoplasm is encroaching on the nuclear cavity.

Fig. 46. ( $\times 800$ .) A tri-polar spindle found near the apex of the egg in the position commonly occupied by the second sperm-nucleus.

Fig. 47. ( $\times 800$ .) Following Fig. 45, and showing the origin of the first segmentation-spindle, which arises between and among the two groups of chromatin. The spirems have mostly segmented at this stage, but the segments have not yet become homogeneous. The chromatin of the egg does not all appear in this section. A preparation showing the two groups of chromosomes to better advantage was injured so that it could not be satisfactorily figured. The spindle is at first multipolar, but soon becomes monaxial, the position of the poles apparently being determined by collections of a dense granular substance which takes a diffuse reddish stain with the Flemming combination. The contents of the



nucleus are more fibrous than before, and the nuclear membrane has disappeared, though the limit of the nuclear cavity remains the same.

Fig. 48. ( $\times 800$ .) The spindle is now monaxial though not yet distinctly bipolar. The chromosomes are homogeneous, and no longer separated into two groups. The contents of the cavity remain as shown in Fig. 47.

Fig. 49. ( $\times 800$ .) This figure apparently represents a later stage than that shown in Fig. 48, but no trace of a spindle could be found, even in very deeply stained preparations. It must either be considered as a cross-section in which the spindle fibres are, for some reason, not easily observed, or the spindle is unusually late in forming.

Fig. 50. ( $\times 800$ .) The spindle of the first segmentation at metakinesis. The division is typical. No centrosomes are present.

Fig. 51. ( $\times 800$ .) Showing a cross-section of the same.

Fig. 52. ( $\times 200$ .) Same as represented in Fig. 50, but showing a section through the entire egg. The division is here oblique, while in Fig. 56 it is represented as longitudinal, and in Fig. 57 as transverse to the axis of the archegonium.

Fig. 53. ( $\times 800$ .) The daughter-chromosomes of the first segmentation approaching the poles in the form of U's and V's. By counting them in several preparations at this stage, their number was found to be twenty-four. The fibres of the central spindle appear twisted and somewhat thickened at the equator, but they soon disappear without the formation of a cell-plate.

Fig. 54. ( $\times 100$ .) The young daughter-nuclei resulting from the first segmentation.

Fig. 55. ( $\times 100$ .) The same at an older stage with the chromatin in the resting condition. The cytoplasm between the nuclei is finely granular. Note the remains of the ventral canal-cell at the apex of the egg.

Fig. 56. ( $\times 800$ .) A spindle of the second segmentation at metakinesis. It is narrower and more pointed than the first segmentation spindle. The chromatin divides in the same manner, and there are no centrosomes present.

Fig. 57. ( $\times 200$ .) A fertilized egg containing two nuclei in simultaneous division. The stage of division is the same as that shown in Fig. 56.

Fig. 58. ( $\times 100$ .) The second segmentation is complete, and the four resulting nuclei are equal in size and without separating walls.

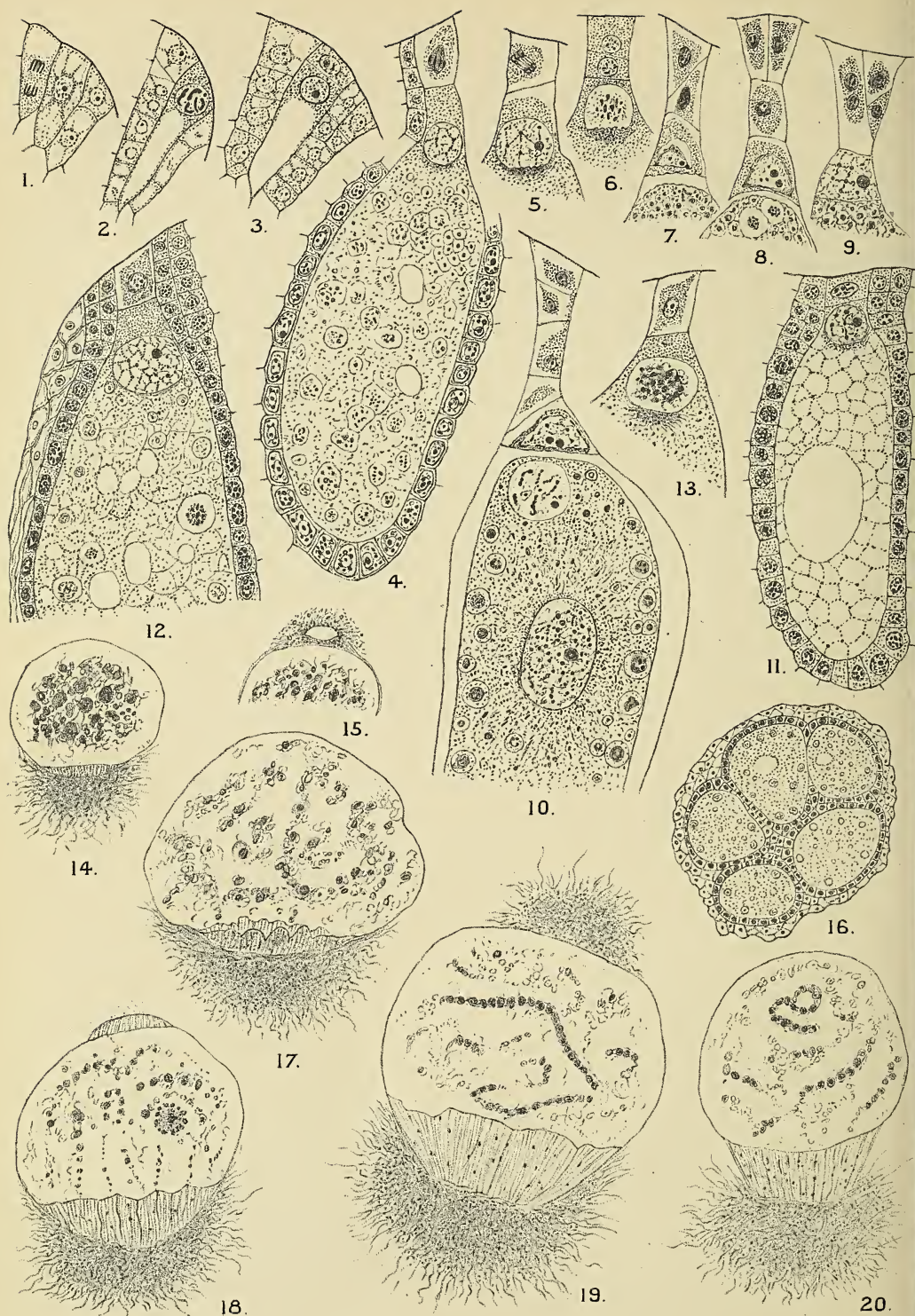
Fig. 59. ( $\times 100$ .) The four nuclei are moving to the base of the archegonium. Their chromatic contents are delicate and scanty. There is no special collection of fibres about the nuclei. The archegonium is cut obliquely so that the position and size of the nuclei are not truly represented in the figure.

Fig. 60. ( $\times 100$ .) The four nuclei have reached the base and are arranged horizontally in one plane. A dense fibrous substance surrounds them. Some distance above the plane of the nuclei is a zone showing a regular reticulum quite free from staining contents. Above this zone the normal contents of the egg are observed deeply stained.



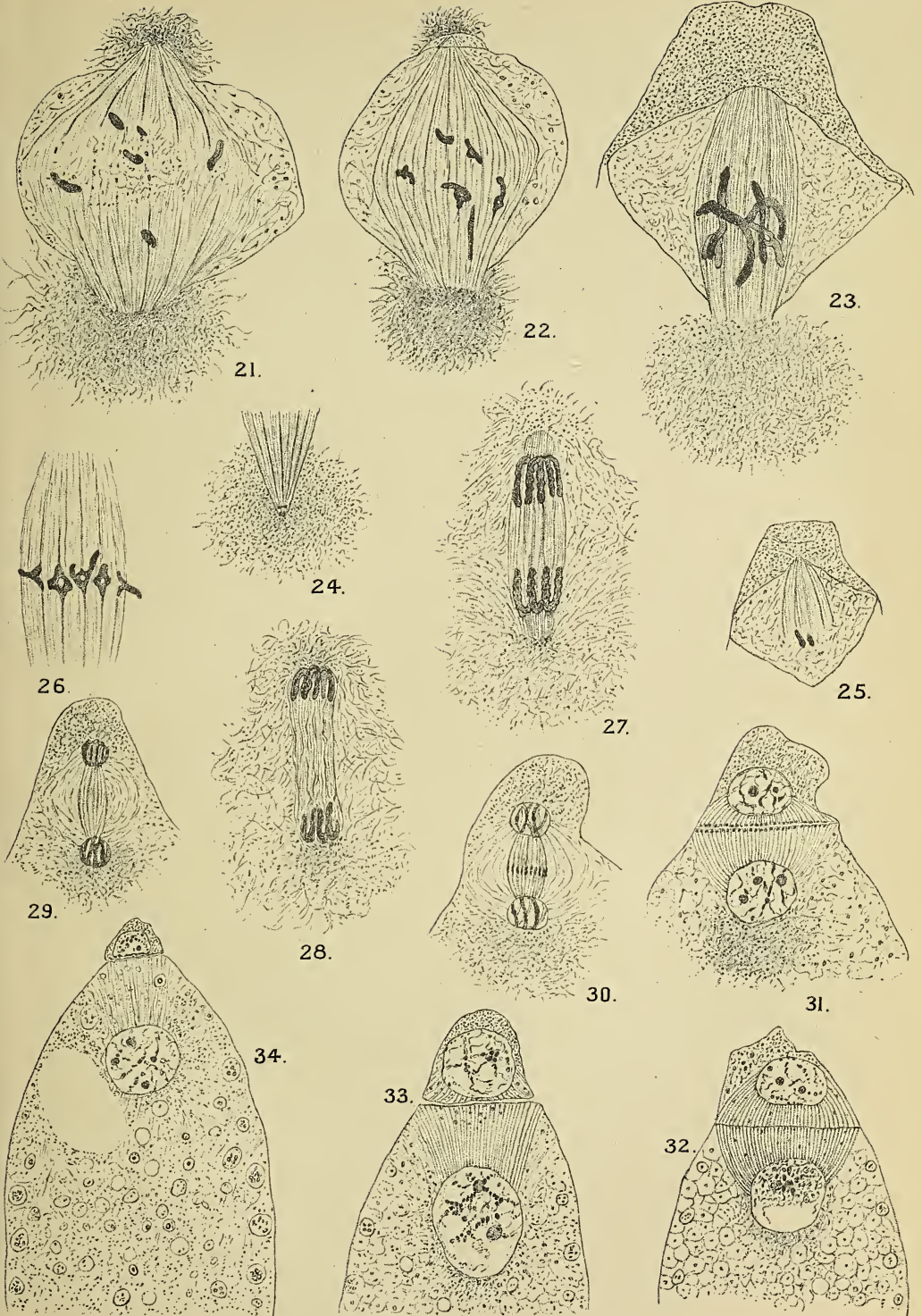






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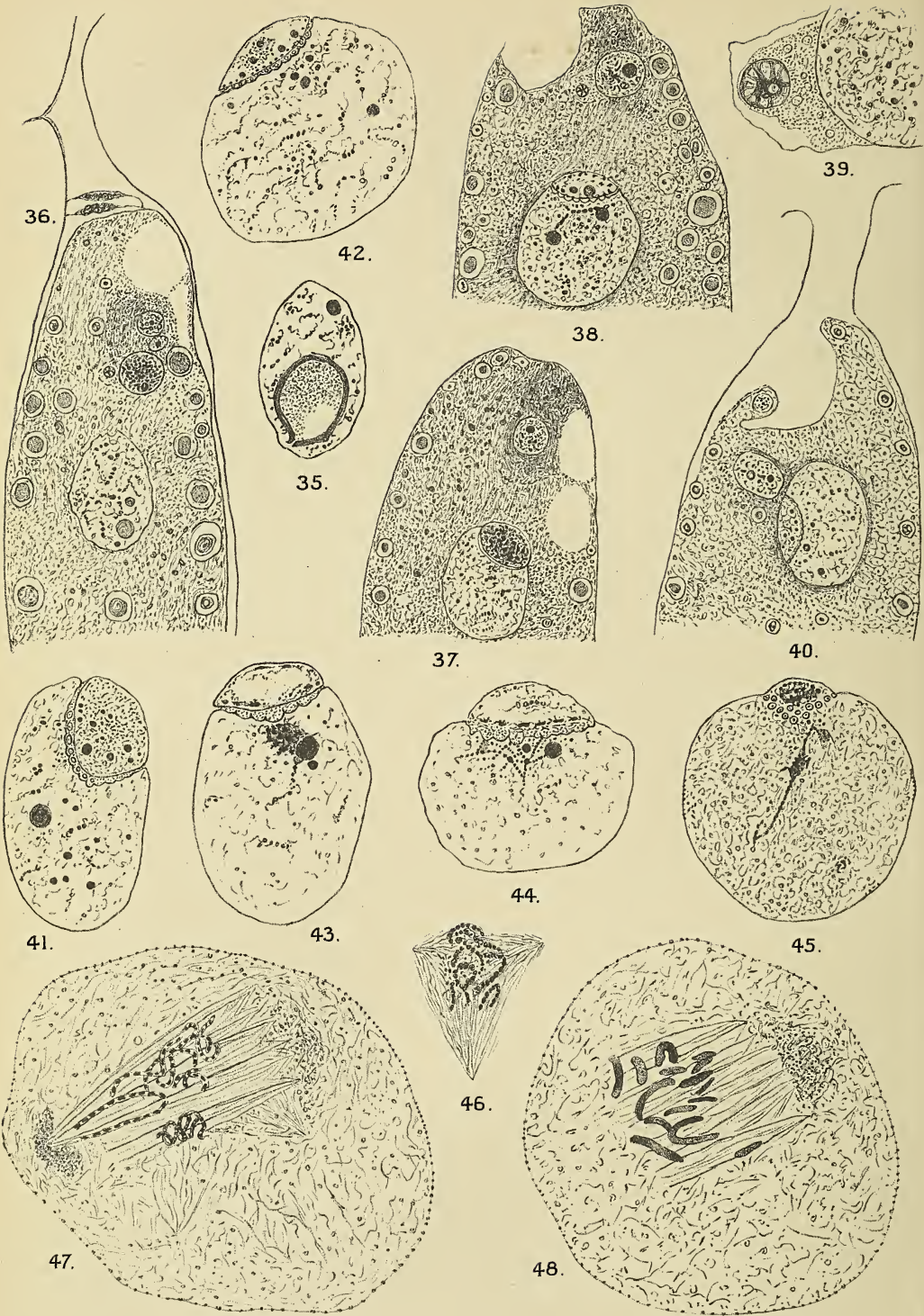






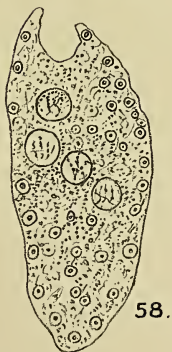
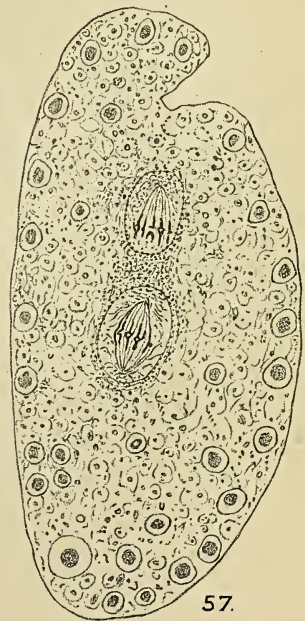
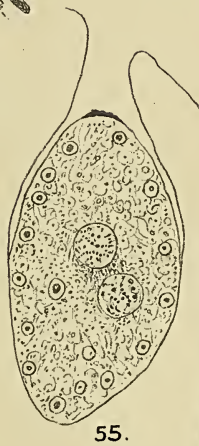
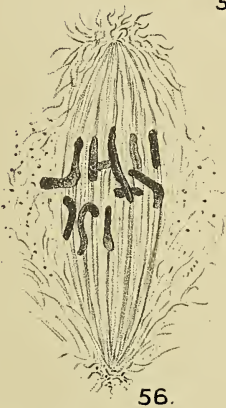
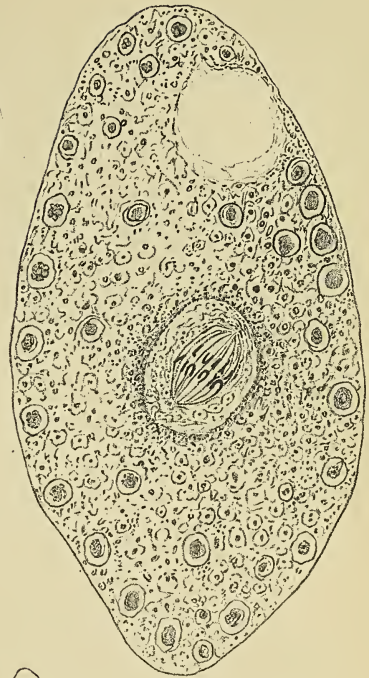
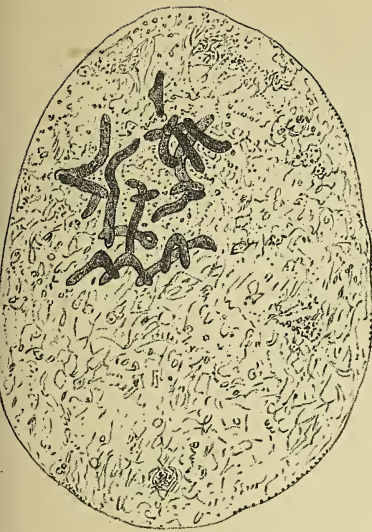






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## Note on the Sugar-cane Disease of the West Indies.

BY

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THE cultivation of the Sugar-cane is still, and—notwithstanding the competition of the Beet in temperate countries—is likely to remain a very important part of the tropical agriculture of the Empire. But of late years it has been hampered, as is sooner or later the fate of all cultural industries, by the ravages of disease.

The problem presented to the botanist in such a case is one of no ordinary difficulty. He has to engage in a conflict with a singularly elusive enemy, and he has to discover the conditions, often by no means obvious, in which that enemy is most open to attack. And the form in which the disease, or the Fungus which produces it, finally presents itself is rarely one which admits of remedial treatment. It is necessary, therefore, to trace back the Fungus through its often multiform life-history, and so to discover the stage at which its mischievous course can be most readily intercepted.

The task is difficult enough when one is face to face with the problem on the spot where it presents itself. It is still more so when the material to be studied only reaches the investigator after a long voyage, more or less decayed and infested with all the Fungi that attend decay:

Nothing is more common than for a Fungus which has long possessed merely a scientific interest and has been preserved in herbaria in scanty specimens, suddenly to exhibit an overpowering fecundity and develop into a scourge.

Something like this seems to have happened in the West Indian cane-fields some ten years ago. A disease made its appearance which caused considerable immediate loss and apprehension of greater.

The disease in Barbados exists in two forms which, though apparently distinct, there is reason to think have a common cause. These are called respectively the 'Rind disease' and the 'Root disease.'

#### RIND DISEASE.

The following account is condensed from the Kew Bulletin, 1895, p. 81 :—Canes infected with the Rind Fungus are first noticed by dark red or brown marks in one or two joints towards the middle or base of the cane. This red patch having made its appearance, rapidly spreads upwards and downwards; the infected area darkens in appearance, and is evidently rotten. Little black specks make their appearance on the cane between the joints, breaking from the inside to the surface; finally the cane shrivels and dries up.

The bursting through of the epidermis is followed by the emission of a black filament, sometimes an inch and a half long or even more. The resulting appearance of the cane is figured by Massee (Ann. of Bot., vol. VII, pl. 27, figs. 1 and 2) and by Prillieux and Delacroix (Bull. Soc. Myc., vol. XI, pl. 10, fig. A). The filaments are composed of agglutinated spores (*Melanconium-stylospores*) which are discharged from a conceptacle or pycnidium buried in the tissues of the internode. This phase of the Fungus was first described by Cooke (Grevillea, vol. XIX, p. 45) from a Queensland specimen as *Strumella Sacchari*.

Massee, regarding it as the conidial stage of a Sphaeriaceous Fungus, named it *Trichosphaeria Sacchari* (Ann. of Bot., vol.



VII, p. 516). The technical diagnosis is given in the Kew Bulletin, 1894, p. 84. Prillieux and Delacroix (l. c., p. 80) identified it with *Coniothyrium melasporum*, Sacc., which is founded upon a specimen from Porto Rico (not Australia), named in manuscript by Berkeley *Darluca melaspora* and described by Cooke. According to Massee (Kew Bulletin, 1895, p. 86), Berkeley's type specimen is a *Diplodia*, and the identification of Prillieux and Delacroix therefore falls to the ground.

All analogy would lead to the conclusion that the life-history of the Rind Fungus comprises more than one reproductive phase. And this proves to be the case. Massee has described the formation of *macroconidia* 'in the interior of a cane, when the tissue is disorganized,' and of *microconidia* on a wounded surface exposed to the air. Both these were obtained in a flask-culture inoculated with *Melanconium-stylospores*, the microconidia being borne on conidiophores growing into the air, the macroconidia being immersed. (Ann. Bot., vol. VII, p. 518.)

Prillieux and Delacroix (l. c., pp. 81, 82) confirm Massee's descriptions of the macro- and microconidia. And generally 'à part quelques points de détail, sans grande importance pratique, ils confirment l'opinion de M. Massee' (l. c., p. 75).

They add still another reproductive stage, that of chlamydo-spores (l. c., p. 81). These have also been observed by Howard.

Went has criticized Massee's results (Ann. of Bot., vol. X, pp. 583-600). His paper was written in Java, where the *Trichosphaeria*, if it exists at all, is 'only to be found on dead canes' (l. c., p. 595). But as Professor Harrison points out (British Guiana Daily Chronicle, Jan. 15, 1897), the work of the Java experts 'appeared to be done chiefly with the white and purple transparent varieties which were relatively immune to some of the diseases affecting the Bourbon,' and Went apparently had not seen the research of Prillieux and Delacroix.

Went thinks that Massee's macro- and microconidia belong to *Thielaviopsis ethacetica*, which produces the 'Pine-apple

Disease.' It is to be noticed that this is not, as might be supposed, a disease of pine-apples, but a disease of the sugar-cane accompanied by a pine-apple odour. He has overlooked the fact that Massee had already called attention to its probable identity with *Trichosphaeria* (Kew Bulletin, 1894, p. 84). Prillieux and Delacroix had done this the following year (l. c., p. 82).

Massee obtained macro- and microconidia in a flask-culture inoculated with stylospores. Went suggests: 'The most probable explanation of this would have been that these macro- and microconidia were an impurity having by chance entered into the flask' (l. c., 594). This seems a purely hypothetical supposition. The experiment has been frequently repeated at Kew with the same result. On the other hand, Went in Java and Howard in Barbados have failed to obtain macro- and microconidia from flask-cultures of stylospores. This, however, does not prove more than that tropical conditions may be unfavourable to their production by this method. Howard, on the other hand, obtained them without difficulty when he inoculated the interior of healthy canes with stylospores, and Prillieux and Delacroix appear to have been equally successful (l. c., p. 81).

It is to be observed that while the stylospores are produced on the external surface of the cane, the macroconidia are only produced in the interior. It is not easy to see how a flask-culture of the former could be accidentally infected with the latter, as suggested by Went.

In any case there can be little doubt that the macro- and microconidia met with in Barbados are actually identical with *Thielaviopsis* (Went, l. c., p. 593). And as Went sought for 'other organs of reproduction' (p. 591), it may be inferred that he regarded this only as a form-genus. A comparison of the figure in Krüger's 'Das Zuckerrohr,' p. 415, of the effect of *Thielaviopsis* on the interior of a sugar-cane with that given by Massee (Ann. of Bot., VII, t. 28, f. 6), showing the growth of the macroconidia of *Trichosphaeria*, will leave little doubt as to their identity.

It may be remarked that Went has ignored the striking resemblance indicated by Massee (l. c., 524) between the macro- and microconidia and their mode of production in *Thielaviopsis* and in *Ceratocystis fimbriata*, Ellis and Halsted (Journ. of Myc., vol. VII, pp. 1-11), which produces the 'Sweet Potato Black Rot.' It is interesting to observe that this has also a pycnidial form, and that as in *Trichosphaeria* the stylospores are extruded in an agglutinated mass.

Ellis and Everhart have briefly described (Journ. Inst. Jam., vol. I, 1892, p. 159) a sugar-cane Fungus under the name of *Trullula Sacchari*. This has been definitely ascertained at Kew to be identical with *Trichosphaeria*. As the diagnosis mentions the 'erumpent' stylospores and the 'catenulate' conidia, it is evident that these authors observed the macroconidia.

According to Went (l. c., p. 595), the *Melanconium* in Java 'is only a saprophyte, and not a wound-parasite, as the form in the West Indies seems to be.' The latter conclusion is, however, abundantly established not merely by the Kew experiments but by Prillieux and Delacroix in Paris (l. c., p. 81) and Howard in Barbados.

It is to be observed that the *Melanconium*-stage of *Trichosphaeria* seems altogether unknown in Java. Its sugar-planters are much to be congratulated. Krüger, who gives (op. cit.) a very full account of all the diseases of the sugar-cane known in Java, indicates nothing in the least resembling the 'Rind Fungus' of the West Indies. What is quite certain is that Went's '*Melanconium* (*Sacchari*?)' has nothing to do with it. Fig. 31 in the Annals (l. c.) would rather suggest that it may be a stage of some Basidiomycetous Fungus. The fourth section of Went's paper (l. c., pp. 595-598) is wholly irrelevant, because it is clear that he has identified under the name of *Melanconium Sacchari* two perfectly distinct things. His experimental results were made with a Javanese Fungus which has nothing to do with *Trichosphaeria*. His results have therefore no bearing on its life-history. He concludes by observing: 'I regret that I am not able to experiment with



*Melanconium* from the West Indies, because I do not wish to introduce this Fungus in the living state into Java (l. c., p. 598).

From a practical point of view the only reproductive form of *Trichosphaeria* of importance is the *Melanconium*-stage producing stylospores. These appear to be ubiquitous in the cane-fields of some of the West Indian Islands, and no attempt seems to be made to destroy canes infested with them. In Antigua Barber says (Kew Bulletin, 1894, p. 176), 'the whole atmosphere is saturated with the spores.' The other reproductive forms of the Fungus appear to be of secondary, at any rate merely of scientific interest. As Massee has pointed out (Kew Bulletin, 1894, p. 83): 'The *Melanconium*-stage can reproduce itself continuously, without the intervention of any other form.' It is 'the conidial form destined for the rapid reproduction and dissemination of the species. . . . The disease is caused by this phase of the Fungus.' The fact is in no way remarkable. In Australia rust in wheat is propagated entirely by the reproduction of uredospores: the æcidial stage is unknown. The 'Leaf Disease' in Ceylon was continued and the cultivation of coffee practically exterminated by the continual reproduction of the uredospores of *Hemileia*.

#### ROOT DISEASE.

About the same time as the 'Rind Disease' a second malady of the sugar-cane, the 'Root Disease,' also attracted attention in Barbados. The following account is taken from the Kew Bulletin (1895, p. 83): 'The canes appear to receive a check in their growth; the plant dwindles down, fresh basal shoots are formed to supply the place of the dying ones, but notwithstanding this it is ultimately found that growth has been arrested and no cane formed: and if the plant be dug up the roots are nearly all dead; and those that are still living are dotted over by little red spots.'

The resemblance of the disease above described to the

'Sereh' of Java has been generally noticed (Kew Bulletin, 1895, p. 83). Went (l. c., p. 588) says it 'looks very much like the "Sereh" in Java.'

It is again to be noticed that 'it was only the Bourbon cane affected. The Caledonian Queen and Transparent are healthy and vigorous.' (Kew Bulletin, 1893, p. 346.)

Diseased stools of sugar-cane were sent to Kew from Barbados for examination. Massee reported that this 'demonstrates conclusively that the disease is due to a parasite fungus known as *Colletotrichum falcatum*, Went' (Kew Bulletin, 1893, p. 347). Went thinks this 'extremely improbable' (l. c., p. 588). He further says that Massee 'gives no evidence for his opinion.' It appears to me, on the contrary, that the description of the Barbados Fungus given by Massee exactly tallies with Went's own description. I do not see what other evidence could be required. And Went (l. c., p. 588) admits having 'received the Fungus from the West Indies.' I may now quote some remarks of my own in the Kew Bulletin (1894, p. 176): 'It is evident that canes infected with "rind fungus" are used for propagation. It further appears that when this was the case the resulting plants are attacked by root disease. This fact points to the conclusion that the root disease and the rind disease are really due to one and the same organism, and that the *Colletotrichum* is only another phase of the polymorphic *Trichosphaeria*. This was indeed suggested by Mr. C. A. Barber, the Superintendent of Agriculture in the Leeward Islands, in a private letter, December 1, 1893, as the result of his observations. But the evidence was not deemed at the time conclusive. The possible identity of the two diseases is still a matter under investigation at Kew.' The Barbados Commission in their Report state: 'It has been finally decided at Kew that *Colletotrichum falcatum*, Went, is simply one phase in the life-history of *Trichosphaeria Sacchari*.' (Kew Bulletin, 1895, p. 83.) This statement was based on information furnished to the Barbados Government but not published. A healthy seedling sugar-cane was inoculated with the spores of *Colletotrichum falcatum*, and at

the end of twenty days developed the *Melanconium*-stage of *Trichosphaeria*. This and the result of other experiments is still open to independent confirmation. But the practical result was of considerable importance. The sugar-cane is propagated by planting 'tops.' It cannot be doubted that these were often infested with the mycelium of the 'rind fungus.' Under these circumstances they failed to develop a healthy cane but, as is believed, exhibited the symptoms of 'root disease.' According to Massee's view (Kew Bulletin, 1894, p. 177): 'The new canes and their rootlets are attacked by the *Colletotrichum*, which, from the evidence at hand, appears to be nothing more than a condition of the *Trichosphaeria*, modified by being more or less buried in the ground.'

Using this as a working theory, the advice was given to take great precautions to avoid planting 'tops' which were possibly infected by rind disease. When followed, the result was 'a marked improvement.' (Kew Bulletin, 1895, p. 88.)

Went (l. c., p. 581) gives the disease produced by *Colletotrichum falcatum*, the name 'Red Smut,' apparently having regard to the red discolouration exhibited by the interior of the affected canes. But the development of a red colour, especially in the neighbourhood of the fibro-vascular bundles, is probably not characteristic of the *Colletotrichum* but may be found in any diseased cane, whatever the cause of the disease.



# On *Trichosphaeria Sacchari*, Massee; a Fungus causing a Disease of the Sugar-cane known as 'Rind Fungus.'

BY

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THE life-history of *Trichosphaeria Sacchari* was first investigated by Mr. George Massee at the Royal Gardens, Kew, and his results are summed up in a paper contributed to the 'Annals of Botany' for December, 1893 (vol. vii, p. 515). I was requested by the Imperial Commissioner of Agriculture for the West Indies to repeat Mr. Massee's work under tropical conditions with a view of ascertaining how far local conditions affect the life-history of the Fungus. The present paper gives an account of the first portion of the work and deals with the *Melanconium*, *micro*- and *macroconidial* stages of the Fungus in so far as the present investigation supplements Mr. Massee's work.

## MELANCONIUM STAGE.

A casual visitor to any cane-field or estate-yard in Barbados during crop time (March to June) is bound to be struck by the large number of diseased canes, locally known as 'rotten-canes,' to be met with. These diseased canes are brown in colour, more or less shrivelled, and in nearly all cases are

[Annals of Botany, Vol. XIV. No. LVI. December, 1900.]

studded with black curly hair-like structures, from a quarter of an inch to two inches or more in length, which are formed under the rind and grow out into the air by rupturing the surface. These hair-like structures, on microscopic examination, are found to be immense numbers of spores of the *Melanconium*-stage of the 'Rind Fungus' loosely cemented together. Extensive cultures of these spores were made under widely different circumstances, the results of which are described below.

Pure Petri-dish cultures of these spores, obtained from a diseased cane, were made in a food-material consisting of raisin-extract containing eleven per cent. of gelatine. The spores developed a white septate branched mycelium which covered the plates as a dense white glistening felt which, as the substratum became exhausted, gradually turned brown, and in fourteen days was found to have developed brown chlamydospores in the hyphae. The nature of these bodies was investigated later and will be described below. Nothing further of interest was observed in this series.

On account of the high temperature of the laboratory, which in the middle of the day sometimes reaches 32°C, it was found that gelatine solutions containing at least fifteen per cent. of that substance are necessary for Petri-dish and hanging-drop cultures. When required for immediate use I find eighteen per cent. gelatine solutions are necessary; when required in a few days' time it is better to prepare the more dilute solution, viz. fifteen per cent., so as to allow for drying up.

Pure plate-cultures were next prepared, using fifteen per cent. saccharose-gelatine containing Klebs' solution:

{	Potassium nitrate . . . . .	2	grams	per	litre.
	Magnesium sulphate . . . . .	1	„	„	
	Potassium phosphate . . . . .	2	„	„	

In preparing this series some of the old mycelium, containing chlamydospores from the above cultures, well beaten up in sterile water, was used for infection. A dense white-felted mycelium was developed as before, in which well-marked

chlamydospores were noted seven days after infection. Similar flask-cultures made at the same time gave similar results. Evidently under these conditions the *Melanconium*-mycelium does not develop in the manner described by Masee in his Kew experiments.

Meanwhile hanging-drop cultures of *Melanconium*-spores were made according to Professor Marshall Ward's method of drop culture<sup>1</sup> in fifteen per cent. saccharose-gelatine containing Klebs' solution. Germination took place twenty-two hours after sowing, and a colourless branched septate mycelium was developed. The day temperature of the laboratory during the development of this series of drop-cultures varied between 26° C. and 29° C. After germination the mycelium developed rapidly, and in two days the drops were filled with mycelium which in many cases showed fusion of the hyphae as described by Marshall Ward<sup>2</sup>. Stages in this process were observed later and will be described below. In three days the mycelium showed extensive vacuolation, and after thirteen days the protoplasm of the mycelium of a single spore-culture showed a tendency to aggregate in certain parts of the hyphae, leaving the rest empty. This proved to be the commencement of chlamydospore formation, stages in which were observed. In twenty-eight days in the drop in question the hyphae were noted to have knotted up together in two areas nearly in contact forming dense stromata. In thirty-four days the edges of the stromata were found to contain *Melanconium*-spores. The stromata were roughly elliptical in shape and measured  $.59 \times .24$  mm. and  $.3 \times .24$  mm. respectively. The *Melanconium*-spores obtained therefrom varied from  $14.7 \mu$  to  $9.2 \mu$  in length and measured  $3.7 \mu$  in breadth. It was unfortunate that the early stages in the formation of these *Melanconium*-stromata were not observed. It was not until after many failures that a hanging-drop culture containing a single *Melanconium*-spore was

<sup>1</sup> The Ginger Beer Plant and the organisms composing it. Phil. Trans., vol. 183 (1892), pp. 130-132.

<sup>2</sup> A Lily Disease. Ann. Bot., vol. ii, p. 329 (1888).



obtained and stages were observed in the formation of a stroma. Working under tropical conditions I have experienced great difficulty in preventing hanging-drops being washed off the cover-glass by condensation of water thereon. This usually happens during the first two days, after which the danger from this source is small.

Next hanging-drop cultures were made in a food-material composed of raisin-extract containing eighteen per cent. of gelatine. Germination took place in one instance in twenty-five hours, in other cases not till forty-eight hours after sowing. Chlamydospores were noted in the hyphae in twelve days, but no stroma was observed in any hanging-drops when raisin-extract was used as a food-material. In flask-cultures, however, where this substance was employed, *Melanconium*-stromata were observed on the surface of the culture in twenty-nine days.

Hanging-drop cultures were next obtained in sugar-cane extract containing eighteen per cent. of gelatine. The cane extract was obtained by boiling small pieces of cane in distilled water for half an hour, filtering the extract and sterilizing in the usual way. When pieces of cane were allowed to soak in water for more than a day it was found that the development of Bacteria rendered the juice so acid that neither *Melanconium*-spores nor vigorous mycelium from other cultures would grow in such a substratum. A hanging-drop containing a single spore, which germinated in thirty-two hours, was obtained. A long colourless hypha was developed which at length branched. In three days the mycelium began coiling up at the point where this branching took place, and from the coil long unseptate hyphae, full of brilliant fine-grained homogeneous protoplasm, radiated in all directions and after a time branched profusely. Subsequent observations showed that these long hyphae were feeders for the coil. The coil rapidly increased in complexity and in five days a stroma was formed. At this point a second smaller stroma was noted in the elliptically-shaped drop, the two being placed approximately at

the foci of the ellipse. In seven days *Melanconium*-conidia were noted at the edges of the stromata. The stromata were elliptical in shape, the larger one measured  $.59 \times .52$  mm. the smaller one  $.54 \times .4$  mm. on the surface of the cover-glass, and both extended about one millimetre downwards. Evidently there is a great tendency on the part of the mycelium developed from a *Melanconium*-conidium to reproduce similar spores. Except as regards time, a natural and an artificial substratum appear to yield the same result, viz. the formation of a stroma on the exhaustion of the substratum from which *Melanconium*-conidia are developed. In this and the other drop-cultures fusion of the hyphae was extremely abundant. Working at Kew with a similar food-material Masee states that he observed no trace of fusion of hyphae in any of the numerous cultures examined when grown in sugar-cane solution<sup>1</sup>. This result is no doubt due to the different method of working adopted and to the different behaviour of the Fungus under Kew conditions. Masee seems to have used hanging-drops only for germination purposes and to have relied solely on flask-cultures for further development. In the host plant, on which *Melanconium*-stromata were abundant, I have frequently observed fusion of the hyphae in the cells near the epidermis, which in many cases are lined with a basket-shaped meshwork of fused hyphae. I have frequently observed similar structures in hanging-drop cultures of *Melanconium*-spores. Perhaps they are the beginnings of stromata which have ceased to grow on account of the exhaustion of the substratum.

A series of plate-cultures with a similar food-material were made at the same time, and here again the formation of *Melanconium*-stromata was observed in great numbers, which after ten days were found to contain *Melanconium*-spores.

Next flask-cultures were made of chlamydospores from previous plate-cultures in fifteen per cent. saccharose gelatine and Klebs' solution, and also in raisin-extract containing

<sup>1</sup> On *Trichosphaeria Sacchari*. Ann. Bot., vol. vii, p. 128 (1893).

fifteen per cent. of gelatine. In sixteen hours a delicate white mycelium was observed with the naked eye at the points of infection and, after a copious development of white mycelium, chlamydospores and *Melanconium*-spores were formed. Evidently the chlamydospores enable the Fungus to tide over an unfavourable period, and explain the remarkable vitality of the hyphae in old specimens of diseased cane observed by Massee.

Up to this point cultures of *Melanconium*-conidia and chlamydospores had reproduced one or both of these spores. Attempts were next made to reproduce Massee's *macro*- and *microconidia* from *Melanconium*-spores grown in cane-juice of varying concentration.

The first series of cultures was made in dilute cane-juice, obtained by boiling slices of ripe cane in distilled water for fifteen minutes, filtering and sterilizing by intermittent boiling, using spores obtained from a pure culture. In three days the flasks were filled with white septate branched hyphae, and in five days dark spots were noted on the matted surface of the culture round the margin. In ten days they were found covering the surface, and microscopic examination showed them to be stromata containing *Melanconium*-spores. The mycelium was found to be very much knotted and to be full of oil drops. I have very frequently noted these oil drops in the mycelium of the Fungus in the host plant, especially near a stroma. No *macro*- or *microconidia* were found in any of this series of cultures after the most careful examination. The submerged mycelium, however, was in many cases found to have divided into free chlamydospores.

The second series of flask-cultures was made in sterilized cane-juice obtained from the clarifiers of a sugar factory. The juice had been heated with lime and gave a slightly alkaline reaction with phenol-phthalein. In seventeen days *Melanconium*-stromata were abundant, but there was no trace of *macro*- or *microconidia*.

Lastly a series of flask-cultures was made in a food-material prepared by boiling slices of very young cane-shoots in dis-



tilled water. In nine days the familiar *Melanconium*-spores were abundant on the surface of the cultures, and the submerged hyphae were found to have divided up into free chlamydospores by the disappearance of the walls of the empty connexions.

Cultures were now made in sterilized cane-slabs by infection with spores from a pure culture. A white mycelium spread out from the point of infection and in six days the mycelium was found to have knotted up in several places forming stromata, which in nine days were found to contain *Melanconium*-spores. At the edges of the cane-slabs the internal mycelium had formed stromata underneath the rind, which grew out into hairs in an exactly similar manner to that noted in naturally infected canes. Similar cultures made on blocks of sterilized oak sap-wood gave similar results. In eleven days the *Melanconium*-spores were abundant on the infected surface.

All attempts to produce the micro- and macroconidial phases of the Fungus in sterilized media had failed.

An extended search in cane fields where 'Rind Fungus' was present and among heaps of rotten canes in estate yards was now made in order to obtain specimens of macro- and microconidia. At length a cane was found, which, on being split open, showed blackening and disorganization of the tissues. The dark colour was found to be due to a copious development of Fungus-spores, which from their size and mode of origin agreed with the micro- and macroconidia described by Massee. Except for the abnormal development of roots at the nodes the cane in question appeared to be healthy till split open. Afterwards similar diseased specimens were obtained from the Marienberg Plantation, Surinam, through the kindness of the manager, Mr. James Mavor. These canes also showed a copious development of roots at the nodes. I have also frequently noted this abnormal root-development in canes which have been badly attacked by the 'moth-borer' (*Diatraea saccharalis*, Fabr.) and by the mycelium of the *Melanconium*-phase of the 'Rind Fungus.' Except in

cases where it is due to rain-water lodging between the leaf sheaths and the stem, it may be regarded as a sure sign of disease. The above specimens were kept in a moist chamber for some time, when they developed *Melanconium*-spores which ruptured the rind.

In view of the occasional development of micro- and macroconidia in the interior of a cane, a further attempt was made to reproduce these phases from the *Melanconium* condition. Two dozen healthy cane pieces were selected about two feet in length, and the cut ends were immediately dipped into hot melted paraffin to prevent the entry of Fungi and Bacteria. The rind of the canes was carefully cleaned and rubbed over with an alcoholic solution of corrosive sublimate. Two series of experiments were made as follows:—

1. Six of the canes were partially split with a sterilized knife and in three of the slits *Melanconium*-spores from a pure culture were sown, while in the other three growing mycelium from a culture of *Melanconium*-spores in sugar-cane extract was introduced. The canes were then lightly bound up with tape and the split end was again dipped into melted paraffin. Six other canes were at the same time treated in an exactly similar manner, except that no spores or mycelium were introduced, to serve as a control experiment.

2. Six canes were split as before and a cavity was made in the cane by cutting out a portion of the interior with a sterilized knife. In three of the cavities spores were sown, while in the other three mycelium was introduced as before. Six other canes were used as a control.

In five days all the canes which had been inoculated with spores were found to exhibit a considerable development of macro- and microconidia near the point of infection. In ten days all the canes which had been inoculated with mycelium gave a similar result. In no case were these spores found in the control canes. In nearly all the canes the split surface was reddish in colour. In nineteen days several of the inoculated canes showed *Melanconium*-pustules on the exterior and blackening under the rind due to the develop-

ment of macroconidia. These two series of experiments confirm Masee's observation that the micro- and macroconidial phases of the Fungus can be obtained from the *Melanconium* condition.

An attempt was now made to obtain a similar result in sterilized cane-slab cultures. Two series of experiments were made, using cane-slabs in the interior of which cavities had been made by splitting the slabs and cutting out a portion of the inside. The two halves were bound up with cotton which had been boiled with water to remove any mineral impurities. In some of these slabs a groove was cut in one of the halves to form a connexion between the cavity and the air. After sterilization, *Melanconium*-spores from a pure culture were sown on the ends of the slabs. In seven days the cane pieces were covered with small *Melanconium*-stromata, and similar structures were found lining those cavities which communicated with the air by means of a groove. In the cavities which had no connexion with the air no spore-formation was noted, although the tissues forming the walls of the cavity were filled with mycelium. In both series no *macro*- or *microconidia* were observed. The day temperature varied from 26° C. to 31° C. during the development of these cultures. In these and other *Melanconium*-cultures on sterilized cane-slabs the red colouration seen in the vascular bundles of living canes attacked by the Rind Fungus was not observed.

Inoculation experiments were performed on healthy canes with *Melanconium*-spores obtained from a pure culture. The spores were placed on the fresh wounds produced by tearing off leaves near the upper part of the cane, and in the small perforations made with a sterilized needle on the internodes of a cane where the rind had been washed and sterilized with an alcoholic solution of corrosive sublimate. After infection the wounds were covered with sterilized vaseline. Other canes were treated in an exactly similar manner, except that no spores were sown on the wounds, to serve as a control. In five days the tissues round the points of infection were



discoloured, and microscopic examination of the tissues at the edges of the discoloured areas showed the presence of hyphae agreeing in all respects with the mycelium of the Rind Fungus. Portions of this tissue taken just under the rind and about half an inch from the point of infection were cultivated in sterile cane-extract. In ten days the mycelium developed therefrom produced characteristic *Melanconium*-stromata. No infection was noted in the control canes. The parasitic character of the *Melanconium*-spores is therefore placed beyond doubt. So far all attempts to inoculate a healthy cane with *Melanconium*-spores without previously wounding the plant have failed.

The result of the above culture experiments with this phase of the Fungus is in close accord with its behaviour in a natural condition. Practically the only spores of the Fungus met with in Barbados are those of the *Melanconium* condition. These are carried by the high trade winds which sweep across the Island during the period when the canes are ripening and when the Fungus is naturally abundant, and bombard the old and young crops. Unfortunately the tunnels of the larvae of the prolific 'Moth-Borer' (*Diatræa saccharalis*) provide the means of entry of these spores right into the heart of the cane. Infection speedily takes place, and when the mycelium has exhausted and killed the host, stromata are formed under the rind from which numberless spores are produced which again spread the disease. The formation of these spores is evidently of more use to the Fungus than the micro- and macroconidia which are formed inside the tissues of the cane, and which therefore would not escape until complete disorganization had taken place.

At the present time little attempt is made in Barbados to keep the Fungus under control. During crop time large piles of 'rotten cane' covered with countless millions of spores are to be found in most of the estate yards of the Island. In many cases these are made into stacks and left for many months, sometimes till the next year. In other cases they are collected by the estate workmen and stored for fuel. In

consequence the yearly loss suffered by the planters from 'Rind Fungus' is very large, and it is obvious that these losses will be incurred in the future unless universal action is taken to destroy the spore-laden rotten canes as quickly as possible by burning.

#### MACROCONIDIAL STAGE.

All cultures of *micro*- and *macroconidia* in liquid media were made in sugar-cane extract which was in some cases stiffened with from fifteen to eighteen per cent. of gelatine.

Preliminary cultures of the mixed *micro*- and *macroconidia* found in the interior of a cane were made on sterilized cane-slabs, in order to ascertain if only one Fungus was present. In twenty-four hours the slabs were covered with a white mycelium which in three days turned black through the development of large numbers of *microconidia* and a few *macroconidia*. Careful examination of these cultures did not disclose the presence of any other Fungus. To test this supposition six plate-cultures were made, using these spores for infection. The plates were speedily covered with a white mycelium, which in two days developed *micro*- and *macroconidia*. The plates were kept under observation till the substratum dried up, but no other Fungus was observed. These plates were used for the following cultures.

A series of flask-cultures was now made, using spores from the above plates. In twenty-four hours the flasks showed a considerable development of a submerged colourless mycelium, which in forty-eight hours darkened on account of the formation of large numbers of *micro*- and *macroconidia*. On account of this extremely rapid spore-formation it appeared desirable to grow the *macroconidium* in hanging-drops and to study the development of this stage of the Fungus starting from a single *macroconidium*.

On account of the simultaneous development of both *macro*- and *microconidia* in all the cultures obtained, some difficulty was experienced in obtaining a drop containing a single *macroconidium* unaccompanied by *microconidia*.

The required culture was at length obtained by beating up some mycelium from a flask-culture, in which micro- and macroconidia were developed in about equal numbers, with sterile water, pouring off the upper portion and using some of the remainder to infect a gelatine-tube. The macroconidia being larger and heavier, subsided more quickly than the lighter microconidia, and therefore the lower layers of water were richer in the former bodies.

The spore germinated in five hours after sowing, and in eleven hours the hypha commenced to branch. In eighteen hours the drop was filled with a branched septate colourless mycelium which exhibited very rapid growth. In twenty-four hours some of the hyphae commenced to grow down into the air, especially round the margin of the drop. This behaviour soon became general all over the drop. The aerial mycelium appeared olive in colour and grew with great rapidity. One of these aerial hyphae was fixed and stages in its development were obtained under the high power. Three hours after leaving the drop the hypha commenced to bend and the contents showed segmentation. Soon after this appearance the hypha became top-heavy and fell back on to the surface of the drop. This was the signal for the rapid liberation of a chain of greyish rectangular conidia, averaging  $7 \times 10 \mu$ , from the distal end of the aerial hypha. This behaviour of the aerial hyphae was found to be general, and except round the edges, the surface of the drop was speedily covered with ejected spores. The aerial hyphae proved to be microconidiophores and the spores microconidia. When first extruded from the hyphae the conidia are greyish in colour, rectangular in shape and filled with granular protoplasm. In an hour after extrusion from the conidiophore the protoplasm of the conidium became vacuolated and the conidia became rounder and larger. After this they gradually turned brown and in twenty-four hours became reddish-brown with a darker coloured central portion, and measured  $15$  to  $10 \mu \times 9$  to  $7 \mu$ . The conidiophores measured  $300 \mu$  or more in length.



During the above developments the submerged hyphae were observed to form short branches from which chains of conidia, larger and darker in colour than the microconidia, were produced. Several likely portions of mycelium were fixed, and stages in the formation of these, which proved to be macroconidia, were observed. The short clavate hyphae soon showed the formation of a clear band near the apex which divided off the protoplasm of the globose end from the remainder. Five minutes later the cell-wall at the apex of the hypha disappeared and a spherical mass of granular protoplasm was extruded. A distinct cell-wall was evident forty minutes after, and the protoplasm was now more coarsely granular and showed vacuolation. After this more conidia were formed in basipetal succession in a chain. Their protoplasm became vacuolated and the walls gradually darkened. When first extruded the protoplasm is finely granular, about thirty minutes afterwards several small vacuoles appear which gradually approach the centre and coalesce. After the formation of the central vacuole the wall begins to darken, and in twelve hours becomes sooty-black in colour, when the central vacuole can no longer be observed. They measure on the average  $22 \times 15 \mu$ .

In many cases in this drop submerged macroconidiophores and collapsed aerial microconidiophores were seen to be developed from the same hypha, thus bearing out Masee's statement that micro- and macroconidia are developed from the same mycelium. The drop in question was freely exposed to the diffused light of the laboratory, but not to direct sunlight, consequently darkness is not necessary for the formation of macroconidia. A similar development was observed in several other hanging-drops containing macroconidia only.

Inoculation experiments were performed on healthy canes, using a mixture of micro- and macroconidia obtained from a pure culture. These were placed on a fresh wound made by tearing off a growing leaf, and also in wounds made in the cane with a sterilized needle. Neighbouring canes were treated in an exactly similar way, to serve as a control. In

all cases the wounds were covered with sterilized vaseline to prevent the entry of other spores. In eight days the tissues of the cane for some distance round the point of infection were much discoloured, and microscopic examination showed that the cells were filled with mycelium which passed from cell to cell by means of pits in the walls after the manner described by Masee. Cultures made of portions of this discoloured tissue showed the copious development of micro- and macroconidia in two days. The control experiments showed no infection.

#### MICROCONIDIAL STAGE.

It now became necessary to obtain a single spore-culture of this phase of the Fungus and follow out the development as in the case of the macroconidia above.

A hanging-drop culture was obtained containing a single microconidium. The spore germinated six hours after sowing and sent out a colourless septate hypha which soon branched. The mycelium quickly extended right through the drop, and its subsequent development was similar in all respects to that described above in the case of the macroconidia. Aerial microconidiophores were formed as before which ejected chains of microconidia, while the submerged hyphae formed chains of macroconidia inside the drop.

The number of microconidia formed by one conidiophore is frequently very large, as many as ninety being observed.

The development of the micro- and macroconidia is therefore practically identical; had the drops not been labelled it would have been impossible to have distinguished between them.

In several cases the microconidia in this and other drops were noted, when a few days old, to germinate in the drop in a curious manner. A narrow hypha was produced  $180\ \mu$  or more in length, into which the brown contents of the microconidium passed, leaving the conidium empty and colourless.

The darkening of the conidia is therefore due to a change in colour in the spore-contents and not in the spore-wall. Possibly the change in colour noted in the macroconidia is due to the same cause. The hypha now proceeded to divide up by transverse walls commencing at the distal end, and the segments which were from 10 to 15  $\mu$  in length and 2 to 3  $\mu$  in breadth now proceeded to round themselves off into a chain of spores. No further changes resulted.

In many of the drops the old brown mycelium was seen to enter on a second phase of growth by sending out very narrow, colourless unseptate hyphae which rarely branched. After a time the growing end passed down into the air as a conidiophore which formed microspores in the ordinary way.

#### ASCIGEROUS STAGE.

Extended search on diseased canes and in old cultures of macroconidia has up to the present not been rewarded by the discovery of perithecia resembling those described by Massee. I am preparing a series of cultures of macroconidia in large flasks with the hope of obtaining perithecia and following out the development of the ascospores.





# A New Type of Transition from Stem to Root in the Vascular System of Seedlings.

BY

ETHEL SARGANT.



With Plate XXXIII.



FOR some years I have been studying the comparative anatomy of Monocotyledonous seedlings, and I have paid particular attention to the transitional region in which the vascular system of the stem assumes the characters of a root-stele. This subject is the more interesting, as the anatomy of that region has as yet been described in few species of Monocotyledons. M. Gérard<sup>1</sup> describes it in nine Monocotyledons, and states expressly that his choice of material was limited because he was obliged to pick out species with comparatively long transitional regions. This is unavoidable when hand-sections only are prepared; with the microtome very few seedlings are unmanageable.

In the Wild Hyacinth (*Scilla festalis*, Salisb., *Endymion* or *Agraphis* of some authors), for instance, the transitional region is not only short but its symmetry is confused by the early formation of a vascular girdle from which the first cauline roots are given off. I have found it impossible to trace the behaviour of each stem-bundle during the transition with complete certainty in this species, although I have four perfect series of microtome sections cut through the critical regions of four well-grown seedlings at the right age.

Such cases, however, are exceptional, and so are those

<sup>1</sup> Gérard, Recherches sur le passage de la racine à la tige. (Ann. des Sc. Nat., Bot., 6<sup>e</sup> sér., t. xi, 1881.)

in which any serious difficulty of manipulation arises. As a rule it is a simple matter to make out the facts in good material at the right stage of growth.

In the second French edition of his 'Traité de Botanique,' M. Van Tieghem has described three types of transition from a stem to a root-structure in the primary axis of Angiospermous seedlings (1891, p. 782). Tracing the transition downwards from stem to root, as I am accustomed to do, these types are shortly as follows :—

1. The number of xylem and phloem-bundles in the upper part of the hypocotyl is the same as the number of such bundles in the stele of the primary root. The transition takes place by the branching of each internal xylem-group to right and left of the phloem-group external to it. The protoxylem of each branch turns outwards during the process. The phloem-groups remain *in situ*, and each is now divided from the next by a pair of xylem-branches with external protoxylem. Each pair fuses into a single group, and the root-stele is complete.

2. The number of xylem and phloem-groups in the upper part of the hypocotyl is twice that of the corresponding bundles in the stele of the primary root. The transition takes place by the fusion of the phloem-groups in pairs. The xylem-groups also approach each other in pairs, the protoxylem of each group becoming external during the process. Thus each pair of centrifugal xylem-groups becomes a single group of centripetal xylem.

3. The number of xylem and phloem-groups in the upper part of the hypocotyl is the same as that characteristic of the primary root-stele. The transition takes place by the branching of each phloem-group to the right and left of the xylem-group within it. At the same time the xylem-groups rearrange their elements so that those of the protoxylem become external. Thus each centripetal xylem-group is divided from the next by a pair of phloem-branches. When a new phloem-group has been constituted from each pair of branches, the root-stele is complete.



I worked out the details of this transition in many Monocotyledonous seedlings before hitting on a case which would not fit into one of these three schemes. The young seedlings of *Anemarrhena asphodeloides*, however, belong to a fourth and hitherto undescribed type. In many respects it is the converse of M. Van Tieghem's second type, just as his third is the converse of his first<sup>1</sup>.

*Anemarrhena asphodeloides* is a herbaceous perennial belonging to the tribe Asphodeleæ of the order Liliaceæ. It is native to the north of China, but is occasionally cultivated in Japanese gardens (Benth. et Hook. Gen. Plant., vol. III, p. 782). Professor S. Ikeno has been good enough to send me seeds of this plant among others, from which I was able without difficulty to raise seedlings for my work. I have had no opportunity of examining the mature plant. One young specimen raised from seed is now in its second year. The root-stock is much flattened and almost disk-shaped; from it spring several shoots of very narrow grass-like leaves. This young plant has not yet flowered.

The three seedlings from which my preparations were made were eleven days old. Fig. 1 (Plate XXXIII) represents the largest ( $A_3$ ) and the smallest ( $A_1$ ) of the trio. The plumule in both is completely enclosed within the base of the cotyledon and is embryonic (Fig. 3). This makes it quite clear that the symmetry of the root-stele is determined by the cotyledonary traces only.

The preparations figured are all from a single seedling ( $A_3$ ), and the levels through which they are cut are shown in the outline of  $A_3$  (Fig. 1). The seed-coats have been shed from this specimen, and the tapering apex of the cotyledon is exposed. A hand-section through this region shows two massive bundles with particularly large phloem-groups, symmetrically placed within the oval outline of the section (Fig. 2). These two bundles run down the cotyledon. They are seen with the same orientation in two hand-sections, not

<sup>1</sup> E. Sargent, On a Fourth Type of Transition from Stem to Root Structure (Report, British Assoc., Bradford, 1900).

figured here, taken about the level marked by a dotted line through  $A_3$  in Fig. 1. The outline of the section is still oval, but a slight groove marks the inner surface of the cotyledon.

The preparations drawn in the four following figures (3-6) are from a microtome series through the region marked by a bracket in the outline of  $A_3$  (Fig. 1). The first (Fig. 3) passes through the embryonic stem-bud enclosed in the sheath-like base of the cotyledon. Besides the two massive bundles of the cotyledon, the section shows three procambial strands in the first leaf. The section drawn in Fig. 4 is .34 m.m. below this. The plumular traces, still embryonic in character, are entering the stele formed by the cotyledonary traces. This marks the first node of the young axis, and the upper limit of the hypocotyl proper. The two lateral plumular traces ( $B$  and  $C$ ) are in the act of joining the stele. The plumular midrib  $A$  has divided, and its branches follow  $B$  and  $C$  respectively. The transition to a root-structure has already begun in the cotyledonary stele by the division of each large phloem-mass. Each cotyledonary trace has now a pair of phloem-groups, and a single wedge-shaped mass of xylem with well-marked internal protoxylem (Fig. 4).

The fusion of the plumular traces with those of the cotyledon is complete at the level of Fig. 5, which is drawn from a section cut .3 m.m. lower than that drawn in Fig. 4. In this section (Fig. 5) the protoxylem-group of each cotyledonary trace has divided into three parts, each of which is on the way to become external. The medium protoxylem group of each trace ( $px_1$ , and  $px_4$ ) will ultimately divide the two branches of each phloem-group from each other. That is,  $px_1$ , when completely external, will divide  $Ph_1$  from  $Ph_2$ , and  $px_4$  will divide  $Ph_1'$  from  $Ph_2'$ . The two lateral protoxylem-groups of each trace will occupy the space intermediate between the two traces;  $px_2$  and  $px_2'$  dividing  $Ph_1$  from  $Ph_1'$ , and  $px_3$  with  $px_3'$  dividing  $Ph_2$  from  $Ph_2'$ .

This process is almost complete in the section drawn in Fig. 6, which is .35 m.m. below that drawn in Fig. 5. All

the protoxylem-groups are external. The lateral groups  $px_3$  and  $px_3'$  have fused with each other. The lateral groups  $px_2$  and  $px_2'$  are on the point of fusing. The stele is already practically that of a root, and is surrounded by an endodermis (Fig. 6).

It is to be regretted that the three series of sections which have been cut through the hypocotyls of three separate seedlings have all been more or less damaged in preparation. The thin-walled tissues in all are crushed and even distorted by the embedding processes necessary before cutting with a microtome. This is least conspicuous in the series from seedlings  $A_3$ , and for this reason I have figured sections from this series, but even in this the crumpled cell-walls are unmistakable, particularly when compared with the tissues in Fig. 2, drawn from a hand-section through the same seedling. The behaviour of the xylem and phloem-bundles is, however, identical in the three seedlings examined, and the transition takes place in each with diagrammatic precision.

The characteristic feature of this transition is that two bundles only enter the hypocotyl which passes into a root with tetrarch symmetry from the beginning. In the second type described by M. Van Tieghem (l.c.) the number of phloem or xylem-bundles entering the hypocotyl from above is twice that of the corresponding bundles in the primary root. This is a common structure among Dicotyledons, as in many Crucifers, but I have not yet found it among Monocotyledons. The fourth scheme, of which *Anemarrhena* is the type, is the converse of this. The stem-bundles entering the hypocotyl are only half as many as those forming the primary root.

A similar structure, but much confused by the presence of plumular traces in the stele of the hypocotyl, and by various irregularities during the transition, is found in the allied genera *Asphodelus* and *Asphodeline*.



## EXPLANATION OF FIGURES IN PLATE XXXIII.

Illustrating Miss E. Sargent's Paper on A New Type of Transition from Stem to Root in the Vascular System of Seedlings.

Fig. 1. Outlines of two of the seedlings cut ( $A_1$  and  $A_3$ ). The apex of the cotyledon is still hidden within the seed in  $A_1$ . The numbered lines drawn through the outline of  $A_3$  refer to the following figures; thus the hand-section drawn in Fig. 2 was cut about the level marked 2 in the outline drawing. Life size.

Fig. 2. Hand-section through upper part of cotyledon ( $A_3$ ), showing the two massive bundles within it. The orientation of this section does not correspond with that of the series from which Figs. 3-6 were drawn.  $\times 75$ .

Figs. 3-6. Four successive sections from a microtome series through the region marked 3-6 in Fig. 1. The orientation is uniform in all, and the lettering as follows:—

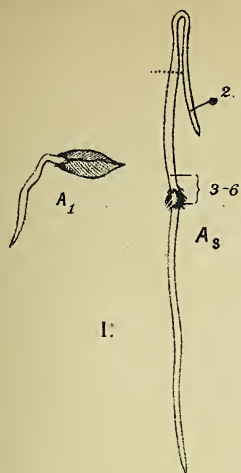
$M, M'$	. . . . .	cotyledonary bundles.
$A$	. . . . .	midrib-trace of first leaf.
$Ph_1, Ph_2$	. . . . .	branches of phloem-group belonging to bundle $M$ .
$Ph_1', Ph_2'$	. . . . .	branches of phloem-group belonging to bundle $M'$ .
$px_1, px_2, px_3$	. . . . .	protoxylem-groups derived from bundle $M$ .
$px_1, px_1, px_1$	. . . . .	protoxylem-groups derived from bundle $M'$ .

Fig. 3. Base of stem-bud, showing embryonic first leaf and growing point completely enclosed within base of cotyledon.  $\times 75$ .

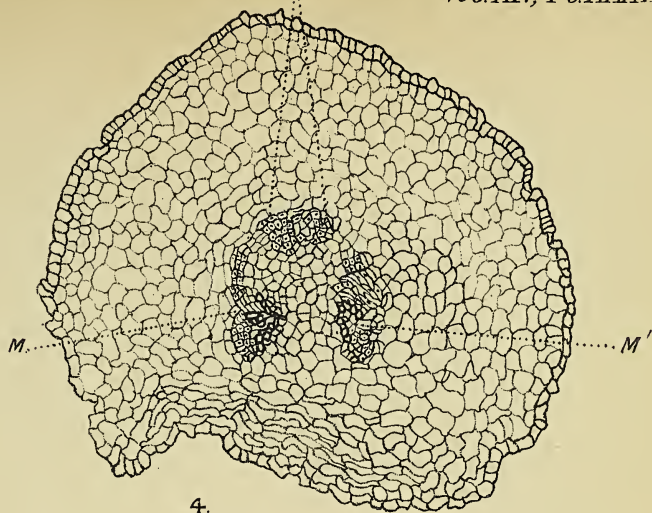
Fig. 4. .34 m.m. below Fig. 3. Lateral traces of first leaf are fusing with  $M$  and  $M'$  respectively. Midrib-trace ( $A$ ) is dividing and will shortly follow the lateral traces. The phloem-groups of  $M$  and  $M'$  have each divided; the protoxylem-groups are as yet undivided and external.  $\times 75$ .

Fig. 5. .3 m.m. below Fig. 4. Central cylinder only, more highly magnified. Plumular traces have fused with cotyledonary traces. Three groups of protoxylem in each cotyledonary trace, becoming external.  $\times 200$ .

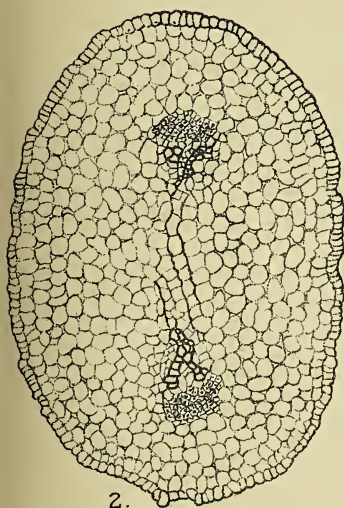
Fig. 6. .35 m.m. below Fig. 5. Transition nearly over. The central cylinder is surrounded by an endodermis. Three external groups of protoxylem are *in situ*; the fourth is not yet complete ( $px_2, px_2'$ ). Four groups of phloem alternate with the xylem-groups.  $\times 200$ .



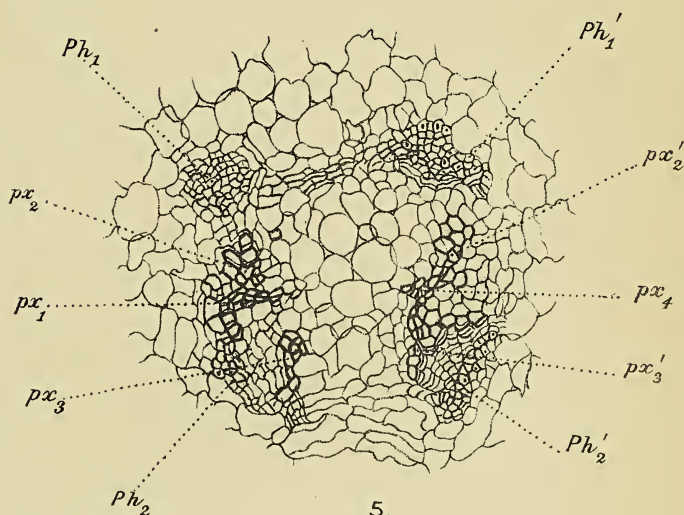
1.



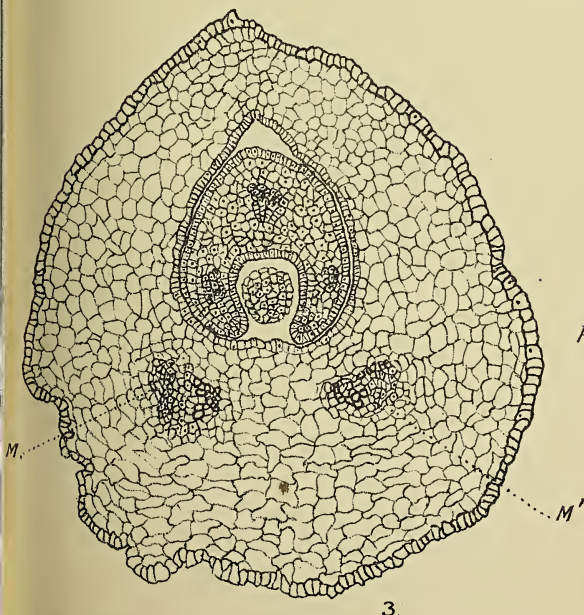
4.



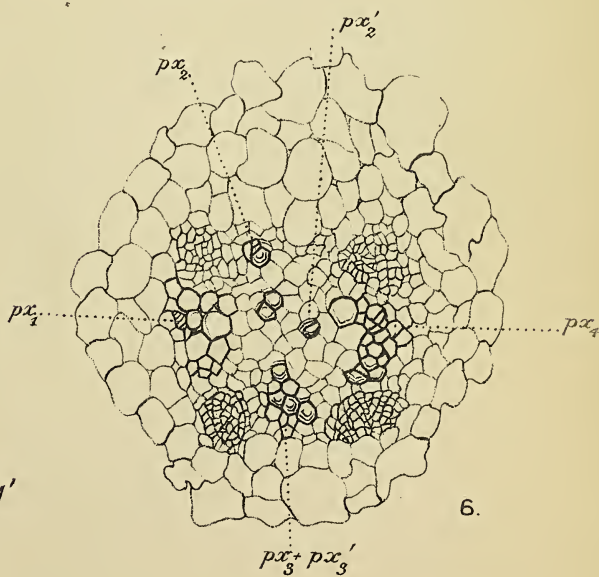
2.



5.



3.



6.





# On the Stem-Structure of *Actinostemma biglandulosa*.

BY

WILLIAM WALLACE, B.Sc. (St. And.).

—♦—  
With Plate XXXIV.  
—♦—

## INTRODUCTION.

THE following are the main features of interest in the anatomy of this stem :—

1. The vascular bundles are primarily collateral and remain so for a relatively long period. It is not until secondary thickening has made considerable progress within the bundles that these begin to acquire medullary phloem.

2. At the base of the older stem are a number of accessory bundles. These have a longitudinal course in the cortex and proceed from a meristem which has its origin in the *endodermis*.

The plant investigated was reared in one of the plant houses of the Royal Botanic Garden, Edinburgh. Belonging to the family Fevilleae-Gomphogyneae of the order Cucurbitaceae (Engler and Prantl), it is a tendriferous liane with an *annual* stem, which, in its upper parts, is slender, cylindrical, and five-angled. A technical description of this species is given in Hooker's *Icones Plantarum* for 1899.

The primary structure, as seen in transverse sections of a young internode, shows the following arrangement of the vascular tissues (Pl. XXXIV, Fig. 1). There are five outer

[*Annals of Botany*, Vol. XIV. No. LVI. December, 1900.]

bundles, each occupying a corner of the five-angled stem. Alternating with these corner bundles and more deeply seated are three larger bundles. On a casual examination these would appear to be the only bundles present at this early stage, namely five outer and three inner; but more careful inspection reveals that there are actually five inner bundles. The remaining two consist each of a few thin-walled, mostly narrow elements, of which those nearer the surface of the stem are differentiated as sieve-tubes with their companion-cells.

At first these bundles consist solely of phloem; later they are collateral with xylem on the inside; finally they become bicollateral, like the rest of the bundles.

One of these imperfect bundles is thicker than the other ( $p$  and  $p'$  in Fig. 1) and develops xylem sooner (Fig. 2). On approaching the node below, the stronger bundle gives off a branch which joins with other bundles to form one of the three large inner bundles of the internode next below, and then, diminished in thickness, proceeds downward through that internode of which it constitutes the thinner imperfect inner bundle. It seems probable that the two bundles just mentioned are the lower portions of leaf-traces: that the larger bundle seen in a transverse section is a trace of a lower leaf cut higher up in its course, while the smaller one is a trace of a higher leaf cut lower down.

#### MEDULLARY PHLOEM.

Most Cucurbitaceae have bicollateral bundles: to this rule, however, the families Zanonieae, Fevilleae, and Gynostemeeae are exceptions<sup>1</sup>. The idea conveyed by the term *bicollateral*, invented by De Bary, is that of a bundle consisting of a mass of xylem between two masses of phloem, all the product of a single procambial meristem. Weiss<sup>2</sup>

<sup>1</sup> Solereder, *Syst. Anat. d. Dicotyledonen*, p. 439, 1898.

<sup>2</sup> Scott and Brebner, 'On Internal Phloem,' *Annals of Botany*, vol. v, 1891, and works there cited.

adopts this conception of bicollaterality, distinguishing medullary phloem from independent strands of phloem by means of the following characters :—

‘1. The medullary phloem-strands in every case accompany the leaf-traces within which they lie, on their exit into the leaf.

2. They arise almost at the same time, or only slightly later than the parts of the phloem outside the xylem.

3. Where a cambium forms in connexion with them, it never produces wood also<sup>1</sup>.’ On the other hand, Hérail<sup>2</sup> declares that in the majority of plants with bicollateral bundles, the medullary phloem does *not* appear at the same time as the rest of the bundle, neither does it proceed from the same meristem. According to him it is only as applied to the bundles of the Cucurbitaceae that the term bicollateral is strictly appropriate; for in *their* case ‘the internal phloem is as primary as the tracheae themselves.’ Lamounette<sup>2</sup> agrees with Hérail that the secondary origin of medullary phloem is the general rule, but denies that the Cucurbitaceae are exceptions to it. From observations of the apex of *Bryonia dioica*, this investigator concluded that the procambial meristem and the meristem which gives rise to medullary phloem are distinct from the first, the medullary phloem being a later development from those conjunctive tissue-cells which abut internally on the procambium.

In *Bryonia dioica*, according to Lamounette, the medullary phloem appears almost, but *not quite* at the same time as the rest of the bundle, and therefore at a point close beneath the growing point of the stem. The primary structure of the stems of species of *Bryonia*, *Trichosanthes*, *Momordica*, and *Melothria* was examined with reference to this particular. In all of them the vascular bundles at this early stage exhibited an abundant medullary as well as external phloem, of which the former was often greater in proportion.

In *Actinostemma biglandulosa*, however, the medullary

<sup>1</sup> This distinction was shown by Scott and Brebner (op. cit.) to be invalid.

<sup>2</sup> Scott and Brebner, ‘On Internal Phloem,’ *Annals of Botany*, vol. v.



phloem makes a late appearance and is never large in amount. The bundles of the seedling are unmistakably collateral (Figs. 7 and 8), as also are those of younger internodes of the older plant (Fig. 9). Sections of a stem over  $\frac{1}{8}$  inch in diameter and a long distance from the apex showed no traces of differentiated medullary phloem (Fig. 9). It is not until a considerable amount of secondary tissue has been produced within the bundles that the elements of the medullary phloem proper to each begin to be differentiated. The appearance of the first sieve-tubes is preceded by the formation of a greater or less amount of small-celled meristem on the inside of the wood of each bundle (Fig. 9). This small-celled meristem arises by division of those cells which abut internally on the protoxylem. The primary sieve-tubes are differentiated at the inner limit of the meristem and form an irregular semicircle around the protoxylem: they are thus separated from the wood by one or more layers of meristem. The further increase in amount of the medullary phloem takes place by means of a medullary cambium formed in the following way:—Cells of the above-mentioned meristem, which lie on the outside of the first-formed sieve-tubes (i. e. nearer the wood), elongate in a direction which is radial with reference to a point occupying the centre of the protoxylem, and divide tangentially with reference to the same point. A somewhat fan-shaped arrangement of cells results (Fig. 10). The inner elements so formed pass over into phloem, but no centripetal wood is formed.

Medullary phloem does not arise simultaneously in relation to all the ten bundles of an internode. The three large inner bundles first acquire it; then the larger of the remaining two inner bundles; next the corner bundles, i. e. those of the outer ring; and finally the remaining inner bundle, which, it will be remembered, was the last to develop its xylem (Figs. 2, 3, 4). As secondary thickening in the bundles proceeds, the more superficial layers of external phloem tend to become crushed against the resistant sclerenchyma. The medullary phloem, on the other hand, experiences no compression,

since the cells of the fundamental tissue adjacent to it are active and make way for its increase. In the case of the large inner bundles this dilatation of the fundamental tissue surrounding them causes the compression of the pith and of the more central portions of the medullary rays.

As in other Cucurbitaceae<sup>1</sup> secondary thickening is at first confined to the bundles: the interfascicular cambium appears late and forms only parenchyma. The tension of the tissues thus set up ruptures the ring of sclerenchyma at points between the bundles, and cells insert themselves into the gaps and subsequently divide.

The bundles remain open throughout the life of the plant. While the normal cambium continues to function, additions are made to the medullary phloem by means of the medullary cambium. The medullary phloem in *Actinostemma* is small in amount as compared with the external phloem, and its sieve-tube elements have only about half the diameter.

Later on, phloem develops at the sides of the wood, which thus comes to be surrounded on all sides by that tissue<sup>2</sup>, as occurs in other Cucurbitaceae.

In *Actinostemma* the medullary phloem does *not* 'accompany the leaf-traces on their exit into the leaf' (contrast Weiss). Transverse sections of the older petiole showed collateral bundles. The medullary phloem accompanies the cotyledonary traces to their lower ends in the hypocotyl, where it stops. The tetrarch or triarch root has no pith and consequently no internal phloem. There is abundance of thin-walled parenchyma in the secondary wood of the root. A search was made for interxylary phloem, such as is described by Scott and Brebner (op. cit.) for the root of *Thladiantha dubia*, but none was found.

<sup>1</sup> De Bary, Comparative Anatomy, English edn., p. 328.

<sup>2</sup> De Bary, Comparative Anatomy.

## ACCESSORY BUNDLES AT THE BASE OF THE STEM.

A number of accessory bundles are present at the base of the older stem. These begin a short distance above the hypocotyl and increase in thickness toward the base of that organ. They are visible externally as prominent ridges, especially on the hypocotyl, giving to transverse sections of this region a very sinuous outline (Fig. 5). The bundles run in the cortex, and there is usually one opposite each primary bundle. They follow the course of the cotyledonary trace-bundles in the hypocotyl, where there is one opposite each of the six bundles in the upper part, and one opposite each of the four bundles in the lower part. The meristem in which these new bundles appear is a product of the layer of cells which lies immediately outside the sclerenchyma. If, according to the statement in De Bary's text-book<sup>1</sup>, 'the ring of sclerenchyma (in Cucurbitaceae, &c.) belongs to the plerome and marks its outer boundary,' then it is evident that we have to deal here with a meristematic *endodermis of a cambial nature*. In its earlier condition the same layer is differentiated as a *starch-sheath*, a fact supporting the above view of its morphological value. This starch-sheath is fairly well marked in transverse sections of the hypocotyl of the seedling (Fig. 6).

Opposite each primary bundle the cells of the endodermis elongate in a radial direction and divide tangentially, giving rise to rows of cells, not unlike periderm, which spread out in a radiate manner from each sclerenchyma-segment (Fig. 12). Between the sclerenchyma-segments, i.e. opposite the primary medullary rays, this formation is interrupted. From a certain number of radial rows a bundle is formed by some of the cells becoming converted into xylem-elements and others into phloem. Between xylem and external phloem the cells remain meristematic and function as a normal cambium, which adds to the thickness of the bundle in the usual manner. In time the wood comes to be surrounded more or less com-

<sup>1</sup> De Bary, op. cit., p. 419.



pletely by phloem, as occurs in the primary bundles. Primary and secondary bundles are connected at intervals by anastomoses which traverse obliquely the medullary rays.

My thanks are due to the Regius Keeper of the Royal Botanic Garden, Edinburgh, who kindly supplied the materials for this work, which, moreover, was done at the Laboratory of the Garden, under his supervision.

## EXPLANATION OF FIGURES IN PLATE XXXIV.

Illustrating Mr. Wallace's Paper on *Actinostemma*.

Fig. 1. Diagrammatic transverse section of a young internode, showing the arrangement and roughly the proportions of the vascular bundles. Xylem light, phloem dark.

Figs. 2, 3, 4. Diagrammatic transverse sections of stem at successively lower levels, illustrating the growth and differentiation of the various bundles.

Fig. 5. Diagrammatic transverse section of old hypocotyl, showing accessory bundles, of which there is one opposite each primary bundle. Shading as before: sclerenchyma brown.

Fig. 6. Portion of a transverse section of hypocotyl of seedling, showing one of four bundles. *E* = starch-sheath or endodermis. The cells immediately within the starch-containing cells will become sclerenchyma. The circular dots are starch grains.  $\times 90$ .

Fig. 7. Portion of transverse section of stem of seedling, showing one of the three larger inner bundles in the primary condition. *ph* = phloem, *px* = protoxylem, *cb* = cambium, *P* = fundamental tissue.  $\times 350$ .

Fig. 8. Portion of a transverse section of hypocotyl of seedling, showing the inner face of one of the six bundles. *px* = protoxylem, *P* = fundamental tissue.  $\times 350$ .

Fig. 9. Portion of a transverse section of an internode about one-eighth inch in diameter, showing the small-celled meristem on the inner face of the wood of one of the three large inner bundles. *px* = protoxylem, *m* = meristem, *P* = fundamental tissue.  $\times 350$ .

Fig. 10. Portion of a transverse section of older hypocotyl, showing inner face of one of the six bundles. *px* = protoxylem, *ph* = phloem, *m. c.* = medullary cambium.  $\times 350$ .

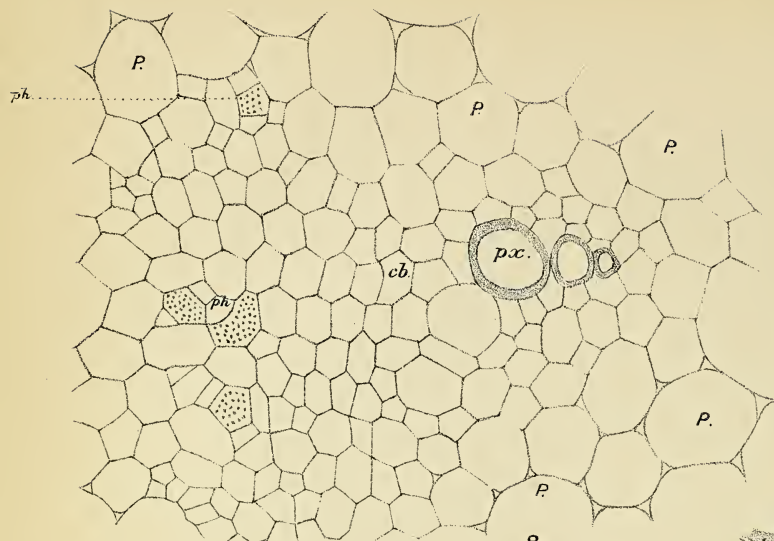
Fig. 11. Portion of a transverse section of stem a short distance above the insertion of the cotyledons, showing division of cells of the endodermis to form the meristem in which accessory bundles arise. *s* = sclerenchyma, *E* = endodermis.  $\times 350$ .

Fig. 12. Portion of a transverse section of older stem above the insertion of the cotyledons, but at a lower level than the section of which Fig. 11 represents a portion; it shows the new bundles in the endodermis. *x* = xylem, *ph* = phloem, *c* = cambium, *m* = medullary ray: all formed from the endodermis. *s* = sclerenchyma, *ph* = the position of the phloem of two primary bundles, *MR* = medullary ray between them.  $\times 90$ .

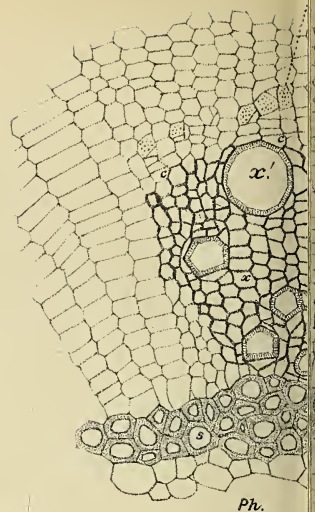




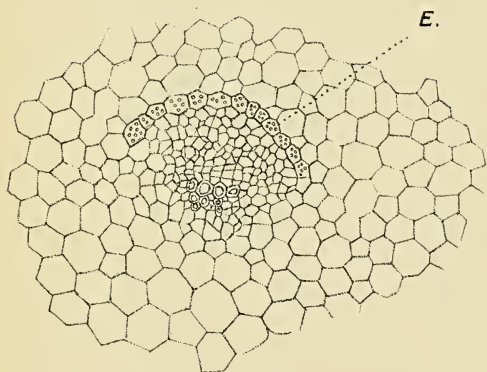




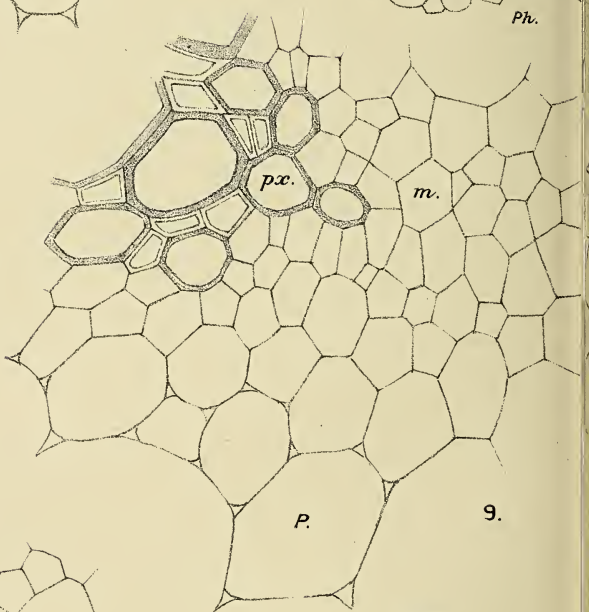
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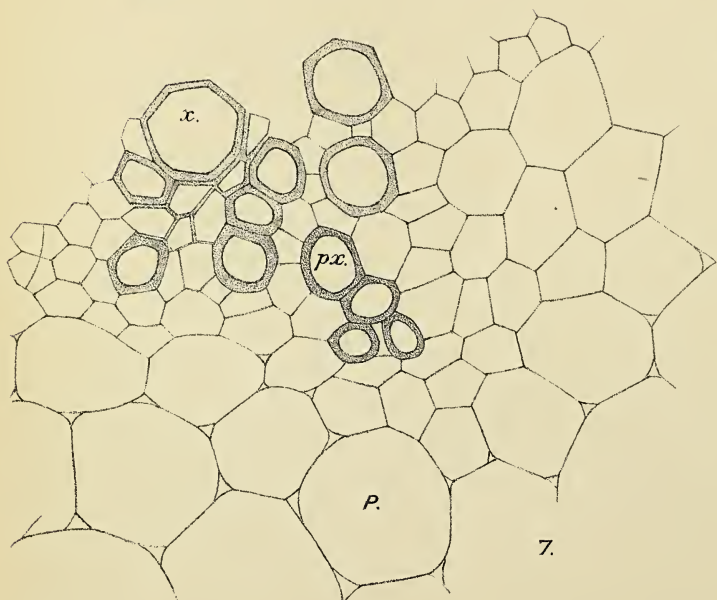
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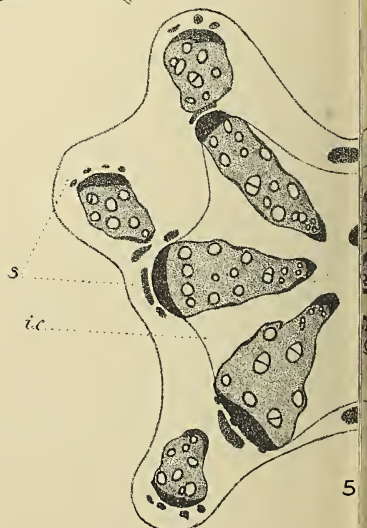
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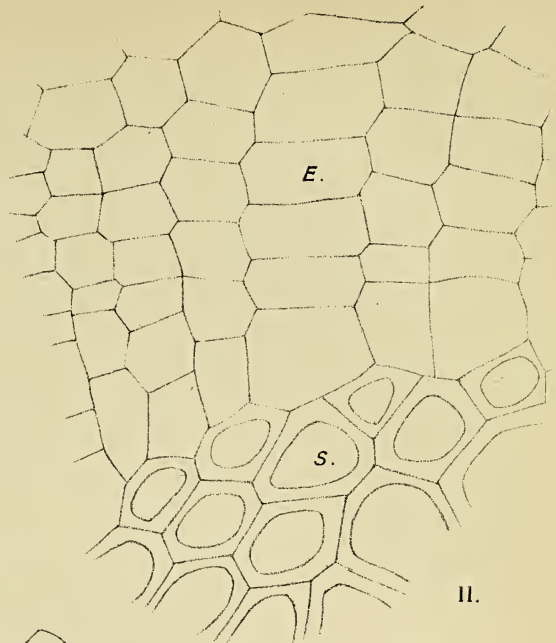
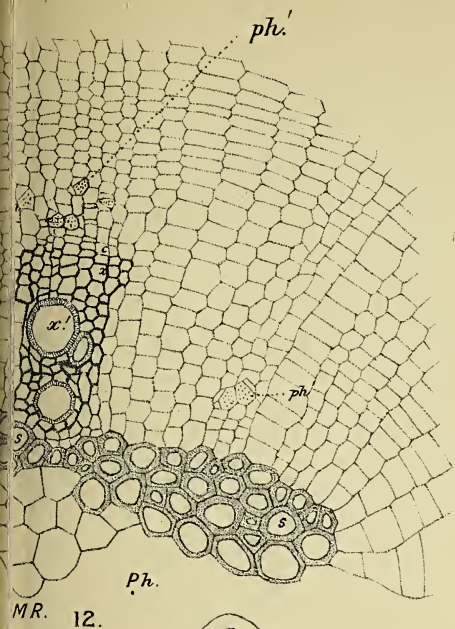
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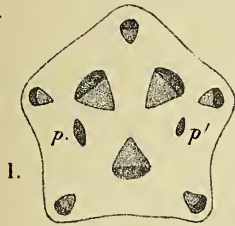
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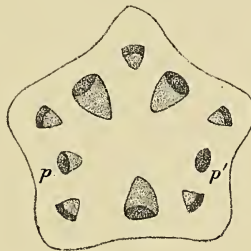
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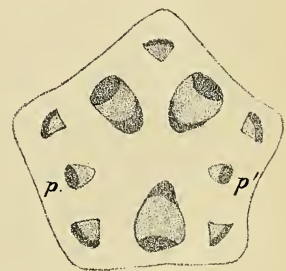
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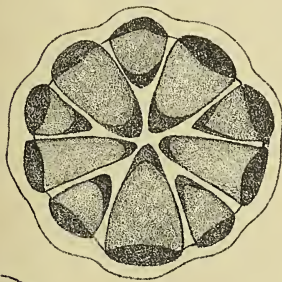
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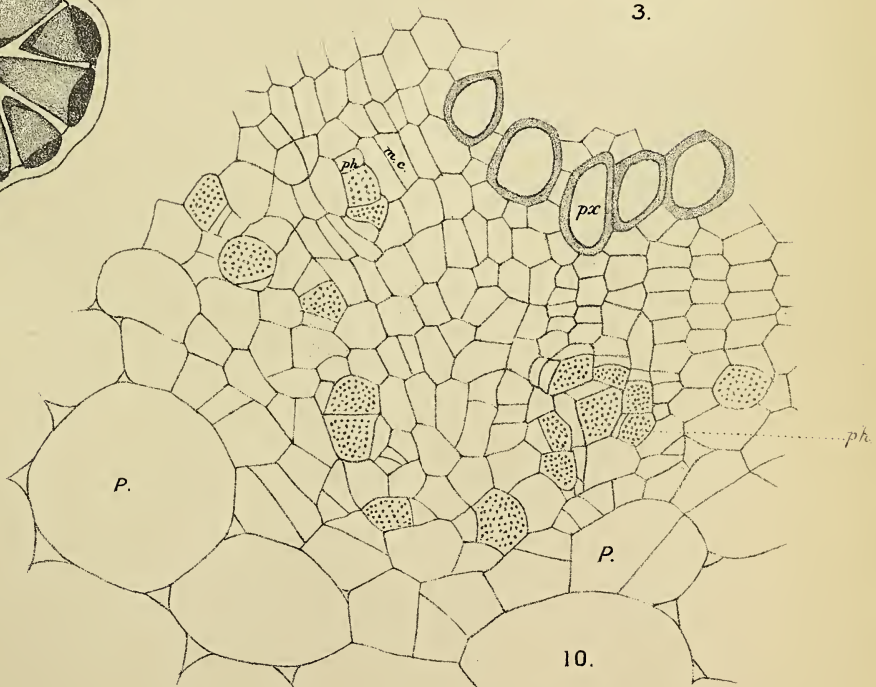
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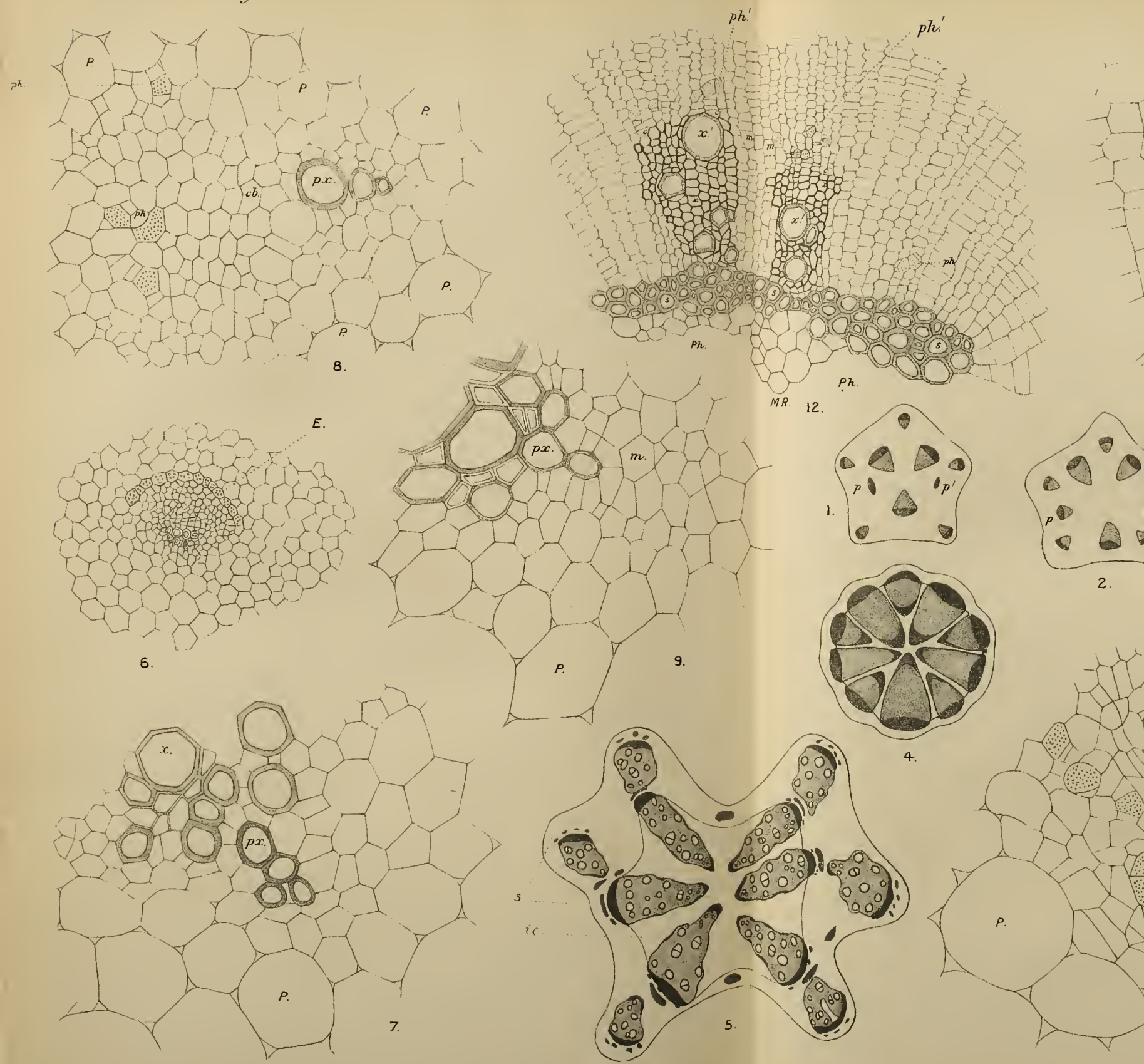
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WALLACE — ON ACTINOSTEMMA.



# The Primitive Algae and the Flagellata. An Account of modern Work bearing on the Evolution of the Algae.

BY

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With two Figures in the Text.



## INTRODUCTION.

**D**URING the last fifteen years our knowledge of the Algae, especially of the green Algae, has made rapid and continuous advance at an increasing rate. As a result we seem now at last to be in a position to grasp something of the phylogenetic relations of what once seemed a chaos of forms, and to correct by the evidence of these vestiges of the early stages of evolution of the vegetable kingdom our conceptions of plant nature and plant possibilities drawn previously only from the study of the higher types.

The publication ten years ago of Wille's account of the green Algae in the *Pflanzenfamilien* of Engler and Prantl ('90) may be held to mark a definite epoch, as his series of articles gathered together and skilfully displayed from the systematic point of view all the knowledge that was then available. This most excellent work has given a great impetus to further investigation, though we have now advanced beyond it in many respects.

The task that I have undertaken is to give some account of the work that has been accumulating on the green Algae

[*Annals of Botany*, Vol. XIV. No. LVI. December, 1900.]



and their allies since Wille's articles were published. In this first article I propose to deal with such of this work as bears on the phylogenetic relations of the primitive forms of green and of brown Algae, and also to give an outline of their respective affinities with the Flagellata.

The literature of this subject is very much scattered, important work coming alike from Scandinavia, Russia, Hungary, and Italy; and as no general account of it has yet been published, I may be allowed perhaps to treat it at some length.

I have endeavoured to weave the different lines of work into one continuous account, so that inevitably I have had to ignore a certain number of opposed theories and to introduce some for which I alone am responsible.

The first section consists of an introductory sketch of the probable inter-relations of the forms which make up the Chlorophyceae, and leads to the conclusion that they are all derived from a *Chlamydomonas*-like organism. The second gives an account of the advance of our knowledge of this important genus, *Chlamydomonas*, during the last ten years. The third inquires into the evolutionary origin of this primary plant type, and contains a sketch of the group Flagellata. The fourth section is devoted to a phylum of green Algae, for which an origin quite apart from that of the Chlorophyceae proper is suggested. The fifth gives a brief account of the recently described primitive forms of the brown Algae, and of their probable evolution also from the Flagellata.

An outline of Wille's classification of the genera with which we shall deal is given on p. 649, and this will serve as a starting-point for our treatment. Forms not mentioned by Wille are added in italics, and those which must now be moved elsewhere are placed in brackets.

The phylogenetic views propounded in the various sections have been worked into one general scheme, which will be found on pp. 684, 685. The lower part is occupied by the Flagellata, and the dotted zone right across includes the forms on the border line, the position of which is still open to question.

An attempt is made to indicate the 'grade' of evolution by position considered vertically, and a horizontal line is drawn across each phylum where it appears to come to an end.

### PROTOCOCCOIDEAE (*Wille emend.*).

1. Volvocaceae	Chlamydomonadeae	{ Chlamydomonas, Carteria, Sphaerella, Chlorogonium
	Phacoteae	Phacotus, Pteromonas
	<i>Polyblepharideae</i>	Polyblepharis, Pyramimonas
	<i>Polytomaceae</i>	<i>Polytoma</i> , <i>Chlamydolepharis</i>
	Volvoceae	{ Gonium, Stephanosphaera, Pandorina, Eudorina, <i>Plaetodrina</i> , Volvox
	<i>Sycaminae</i> ?	<i>Sycamina</i>
5. Protococcaceae	Endosphaereae	{ Endosphaera, Chlorochytrium, Phyllobium, Chlorococcum
	Halosphaereae	Halosphaera
	Characieae	{ Characium [Ophiocytium] [Sciadium] [Peroniella]
6. Hydrodictyaceae		{ Hydrodictyon, Pediastrum [Coelastrium] [Sorastrum]
2. Tetrasporaceae		{ Chlorangium, Tetraspora, <i>Radiofilum</i>
		{ [Mischococcus], <i>Chlorodendron</i> [Dictyosphaerium] [Oocardium]
3. Chlorosphaeraceae		Chlorosphaera
4. Pleurococcaceae		{ Pleurococcus, Scenedesmus, Raphidium [Stichococcus]

### CONFERVOIDEAE.

7. Ulvaceae	Monostroma, Ulva, Letterstedtia	
8. Ulothrichaceae	{ Ulothrix, Hormidium [Conferva]	
	{ [Bumilleria], Microspora	
9. Chaetophoraceae	Chaetophoreae	Stigeoclonium, <i>Pseudopleurococcus</i>
	Chroolepidae	Trentepohlia
	[Phaeothamniae]	[Phaeothamnion]
10. Mycoideaceae	11. Cylindrocapsaceae	12. Oedogoniaceae
13. Coleochaetaceae	14. Cladophoraceae	15. Gomontiaceae
16. Sphaeropleaceae		

### SECTION I. *Chlorophyceae*.

The subdivision of this group which is generally observed is that employed by Wille, into Siphoneae, Confervoideae, and Protococcoideae. The first of these groups is a natural

one based on the coenocytic structure of the thallus, and will probably remain long unchanged. The other groups are not natural, and must before long be abandoned.

The Confervoideae contain those types of organization which are filamentous or membraneous, and which are universally accepted as forming one step in phylogenetic ascent from the lowest unicellular Algae to the higher plants.

The Protococcoideae seem to have been first segregated as a heterogeneous remainder of primitive forms left after the characterization of the other two groups. In the Protococcoideae we find all grades of sexual reproduction up to oogamy (*Volvox*), and many different types of vegetative organization: motile single cells (*Chlamydomonas*), non-motile single cells (*Endosphaeraeae*), loose indefinite colonies (*Tetraspora*), definite coenobia of motile (*Volvox*), or of non-motile cells (*Coelastrum*), and even coenobia of coenocytes (*Hydrodictyon*). Some of these organisms live entirely by vegetative division, and others are characterized by never dividing vegetatively. This chaos of forms resolves itself, when studied inductively, into some ten more or less natural groups (sub-families of Wille); cf. p. 649.

Among this group of families there are three divergent vegetative tendencies, which furnish, I think, the natural broad phylogenetic lines on which to arrange the different types. These I would distinguish as follows:—

The first of these tendencies, the Volvocine, is towards the aggregation of motile vegetative cells into gradually larger and more specialized motile true coenobia.

The second, or Tetrasporine, is towards the formation of aggregations by the juxtaposition of the products of septate vegetative cell-division to form non-motile organisms of increasing definiteness and solidarity.

The third, or Endosphaerine, tends towards the reduction of vegetative division and septate cell-formation to a minimum. The organisms on this line are strictly unicellular and non-dividing when primitive; unseptate coenocytes when more advanced in type.



Let us now examine these three tendencies and the different organisms which manifest them in order to see what their origin may have been and whither they lead.

1. The manifestation of the Volvocine tendency is comprised within the single sub-family Volvoceae (Wille). This consists of a series of genera which are practically coenobia of cells of the Chlamydomonad type (see Section II). The other sub-families of the Volvocaceae, except Scyaminae, are like *Chlamydomonas*, strictly unicellular motile individuals and in the Volvoceae we find evolving from them, by the aggregations of such units, the ascending series of forms of *Gonium*, *Pandorina*, *Eudorina*, *Placodorina*, and *Volvox*. *Volvox* is undoubtedly the highest of these motile coenobia<sup>1</sup>, and has reached a very high degree of organization in that it has parts specialized for reproduction and a true non-reproductive 'soma.' The sexual reproduction is oogamous, and *Coleochaete* alone among the green Algae exhibits a higher method. Along this line of evolution nothing higher than *Volvox* exists, and this tendency comes to a blind end within the Chlorophyceae, or indeed within its lowest group—the Protococcoideae—as generally understood.

In its grade of organization and of reproduction *Volvox* is on a level with high forms in the Siphoneae and Coniferoideae, and is, I think, much too highly evolved to be included in a family of primitive forms. Yet it would be impossible to remove it from the Protococcoideae without taking out also the primitive *Chlamydomonas*, so closely are they linked by intermediate forms. This alone shows how unnatural is this 'grade' group.

2. The second tendency is exhibited in the Tetrastoraceae, where vegetative cell-division is the chief method of multiplication, and in the Pleurococcaceae, where it is the only

<sup>1</sup> By a 'coenobium' is to be understood, structurally, a definite colony of cells which is reproduced as such from a mother-cell, and which never multiplies its number of units when once thus formed, so that all its cells belong to the same generation. Coenobia exhibit very different degrees of solidarity, individuality, and differentiation.

one. In the simplest types the cells so formed separate from one another, and so no advance in organization follows. In higher forms the sister-cells are united by mucilage, by remains of the mother-cell-wall, or in other ways, so that colonies arise.

The Tetrasporine plants are always non-motile in their vegetative condition, and some of the most primitive, as *Chlorangium*, are little more than sessile attached Chlamydomonads which develop by dividing vegetatively into new cells, that remain attached to the mother-cell-wall and build up thus a little colony. Before this reaches any size, however, the building-up stops, and the cells—as zoospores—revert to the ancestral motile type and swarm off to new spots.

Like the Volvocine tendency then, the Tetrasporine has its origin in the motile unicellular Chlamydomonads. The one of these Tetrasporine aggregations that has been most successful from the biological standpoint is the filament, and as transition forms we find filaments (*Stichococcus*, *Bumilleria*, *Hormidium*) which are not yet possessed of much solidarity, and which easily separate into single cells or short lengths. Numerous other forms of colonial cell-unions are found in these families, but nothing higher appears to have been evolved from any of them. These may perhaps be looked upon as experimental types of aggregation which have failed in the struggle for existence. The filament, however, has been eminently a success, and the large group of filamentous and membranous thalloid forms constituting the Confervoideae bears witness to this.

From the Confervoideae, as is universally admitted, has been evolved the whole series of higher plants, Bryophyta, Pteridophyta, and Phanerogamia, so that the tendency seen in the Tetrasporaceae to form, by septate cell-division, thalloid aggregates of increasing solidarity is the one responsible for the structural possibilities of the higher plants.

A line of separation between the Protococcoideae and the Confervoideae, that is the separation of unicellular organisms from filamentous organisms, is found to be untenable now that

investigations on the effect of varying external conditions on the form and function have made such rapid progress.

Klebs ('96) was the pioneer in this as in many other important algological departures, and from his work it appeared that under certain definite conditions filamentous forms may break up into small aggregates or into single cells.

To Klercker ('96) we owe an absolute proof of the truth of this, by observation of two species of the genus *Stichococcus*. Since the early investigations of Nägeli ('49) a unicellular Alga, *Stichococcus bacillaris*, had been recognized, which had the shape and method of vegetative division of a large, thick Bacterium. It has, however, one clear green chloroplast attached to the side of the cell and leaving the two ends colourless. No zoospores, gametes, or resting-cells occur, so it clearly found a place in the Pleurococcaceae. A filamentous Alga with rather similar cell-contents had been variously named as *Ulothrix subtilis* or *Hormidium subtile*, and though nothing was known of its methods of reproduction, its unbranched filamentous form entitled it to a place in the Ulothrichaceae.

The similarity of the cytological structure suggested some affinity between these forms, and Klercker has succeeded in showing that each of them can exist in a filamentous state and a unicellular 'coccoid' state. He proves this by successive drawings under the microscope, showing the individual cells of the filament separating from one another and drifting apart with rounded ends to continue existence and multiplication in the coccoid state (Fig. 14 *a* and *b*). The cytological characters are retained unchanged through the transition, and these two species, *S. subtilis* and *S. bacillaris*, are clearly thus distinguishable.

Normally these two species exist, one (*S. bacillaris*) as separate single cells, and the other (*S. subtilis*) in the form of filaments, so that we have a genus with one species in one main group (Confervoideae), and the other in another (Proto-coccoideae). Nothing could better exemplify the unnaturalness of the distinction.



This easy reversion to a unicellular form shows how the filamentous type may have been evolved by adhesion of the products of vegetative division. The unbranched primitive filamentous Confervoideae are no doubt polyphyletic, and it seems natural to associate the main forms, having zoospores and gametes, with the Tetrasporaceous unicellular type, and forms not having them with the Pleurococcaceous type, as in the case of *Stichococcus*.

Wille ('90) and others represent the membranous Ulvaceae as the root-family of the Confervoideae because the passage from *Tetraspora* to this family is so easy. The derivation of the filamentous Ulothrichaceae from the Ulvaceae seems to me a mistaken idea, when a direct origin for them from those Tetrasporaceae which tend in a filamentous direction is so much simpler. *Radiofilum*, Bohlin ('97), G. S. West ('98), suggests such a form, consisting of a string of cells imbedded in a mucilaginous matrix which has the shape of an unbranched filament (Fig. 14). The Ulvaceae may be regarded, then, as a side branch from the Tetrasporaceae, ending blindly in the highly differentiated form *Letterstedtia*.

The case of *Pseudopleurococcus*, Snow ('98), is another of those in which by change of conditions an Alga that is normally unicellular passes into a branched filamentous state. Here the two forms taken independently would be placed in the branched Chaetophoraceae and in the Pleurococcaceae respectively, which is a wider separation than had been made between the forms of *Stichococcus*.

The characterization of families by external form is, in this group, quite unpermissible<sup>1</sup>, and the present subdivision into families, representing different grades of vegetative morphological complexity, can only be regarded as temporary until our increasing knowledge enables us to form phyletic families.

<sup>1</sup> Senn ('99) has shown that by appropriate conditions the coenobia of some Pleurococcaceae—*Coelastrum*, *Scenedesmus*—can be made to give rise to single isolated vegetative cells which lack the formal characters of the cells in the coenobia. Fig. 14, *Coelastrum reticulatum*, *a*, cells united by mucilage arms as in the coenobium; *b*, armless free cells.

3. We must now return to the third line of evolution. The Endosphaerine tendency is exhibited by the Protococcaceae and Hydrodictyaceae, but we do not find here clear serial arrangement of stages in its development. The family Protococcaceae is an aggregate of diversified forms, and almost all the genera of one sub-family—the Characieae—have been removed elsewhere (cf. Section IV), while *Halosphaera* has a sub-family to itself. In all, however, we find complete absence of vegetative division, and individuals are only multiplied by formation of zoospores or of gametes. The Endosphaeraceae are mostly strictly unicellular, but *Phyllobium* shows some coenocytic tendencies.

The Hydrodictyaceae as revised contain only complex forms, which are coenobia of coenocytes, and never exhibit septate vegetative division; but the affinities of this specialized family with its neighbours are not yet clear. *Coelastrum*, however, is known to multiply its coenobia by vegetative division; but Senn ('99), in a paper, the most important parts of which will be dealt with subsequently, has shown clearly that *Coelastrum* (and with it *Sorastrum*, presumably) must be transferred to the Pleurococcaceae, leaving only *Pediastrum* to be grouped with *Hydrodictyon*. In the family thus restricted the coenobia in both genera arise by the apposition of motile zoospores within a mother-cell-wall, while all the coenobia of the Tetrasporaceae and Pleurococcaceae are formed by vegetative septate division of a mother-cell. Thus the contrast of tendency on these two diverging lines of evolution which I have been maintaining becomes by this rectification still more pronounced.

With very high probability it is this Endosphaerine tendency which, carried further, has given rise to the well-defined type of the Siphoneae, consisting of a thallus which, though essentially coenocytic, is structurally unicellular and lacks the solidity acquired by septate cell-division. The possibilities of this type of aggregation are considerable, and it has given rise to many very varied forms; but as with the Volvocine type, nothing appears to have been evolved from it of higher

status than an Alga. While the Tetrasporine tendency has given rise to all the higher green plants, the Endosphaerine has only succeeded in producing the elaborate but puny mockery of them which we find in *Caulerpa*.

The simplest forms exhibiting this tendency seem to be derived also from the motile unicellular *Chlamydomonas* type by loss of motility and by dependence on zoospore-formation in place of vegetative division, which is in direct opposition to the line of development of the Tetrasporaceae.

All the three lines of evolution which we have now considered seem clearly to diverge from *Chlamydomonas*, and this motile organism must be regarded as the real primitive form of green plant.

Beyond the glamour which this position throws over the Chlamydomonads, an immense importance should be attached to obtaining as complete knowledge as may be possible about the biology, structure, and reproduction of form, in which we have the vegetative potentialities of all the different types of green actualities which have filled the earth and attained justification by natural selection.

## SECTION II. *Chlamydomonas*.

This section will be devoted mainly to the consideration of the genus *Chlamydomonas*. This is so much richer in species, in varieties of life-history, of structure and of reproduction, than the other genera in its family, that many monographs have been devoted to the investigation of it; and as knowledge of the Algae increases, the tendency is always towards emphasizing the importance of this genus.

A typical Chlamydomonad as *Chl. Steinii* (Fig. 13), Goros., consists of a single oviform cell, which swims freely by two cilia projecting from the advancing narrow end. It has a clearly defined cellulose wall, within which is the solid protoplast. The greater part of the cell is occupied by the single large basin-shaped chloroplast, a small region of colourless protoplasm being visible at the anterior end only. To this



specialized non-granular protoplasmic end are attached the cilia, protoplasmic in nature, which perforate the cell-wall. Here also are the two small contractile vacuoles which, pro-

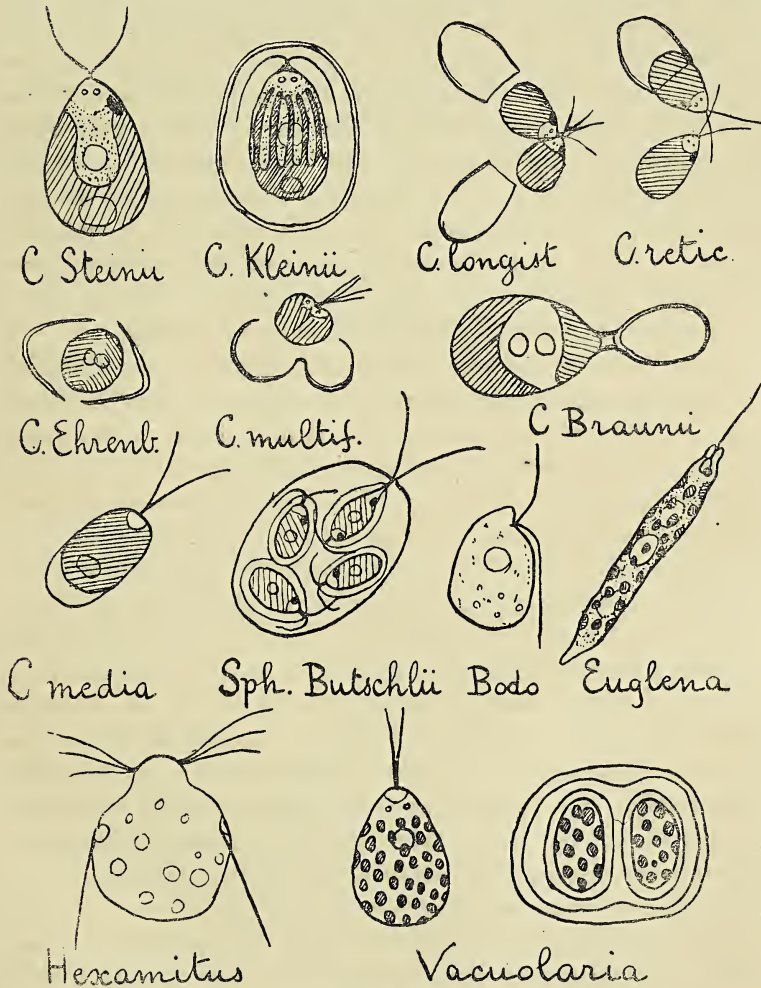


Fig. 13.

bably excretory in nature, contract and recover once a minute or so. The chloroplast contains one large pyrenoid of a proteid nature in the centre of its thick basal part. The

hollow of the chloroplast contains granular protoplasm in which the nucleus is situated. Finally, somewhere on the periphery of the anterior half of the protoplast is a bright red pigmented disk, the eye-spot or stigma, which is in some way associated with the directional reaction of the organism to radiation.

Most genera of unicellular Algae of simple form (in opposition to Desmids and Diatoms) consist of only a very few, difficultly characterized species. Quite the reverse holds with *Chlamydomonas*, but in distinguishing the species, the shapes and sizes of the individuals are of no significance, and the cell-walls show practically no variation: cytological characters are alone available. Yet it appears that at least eighteen good species may be precisely diagnosed in this way.

Wille, in 1890, allowed some six species. Goroschankin ('91) in the next year published an admirable study of the specific characters in this genus, and defined clearly nine species. These were all carefully figured, and the life-history was followed in every case. The characters are so precise that the author is able to draw up a tabular analytical key, by which the different species may be distinguished in their motile vegetative condition. The sort of differences on which this is based may be briefly enumerated. The chloroplast may be solid or reticulate, ring or basin shaped; the contractile vacuoles, two or three in number; the cilia, two or four, short or long; the pyrenoids, absent or single or two, boat-shaped or spherical; the eye-spot shaped like a rod or a disk; and, finally, the zygote may be smooth-walled or spiny. Higher up in the green Algae uniform cytological characters characterize genera and families, but in this primitive type we learn that they only hold for the species.

Francé, in 1892, in a revision of the genus, maintained that these cytological differences were not really constant, and proposed to reduce largely the number of good species. No experimental evidence of the passage of one form into another was, however, presented.

Schmidle, in 1893, described a very interesting new species,

*Chl. Kleinii*. He cultivated this under various different conditions, and found that its well-marked cytological characters remained quite constant. This species is further of interest because it shows less of the characteristic motility in its vegetative condition than any other member of the Volvocineae. It only swims freely for a very short time, never dividing while free, and then comes to rest on some substratum, and surrounds itself with a mucilaginous excretion, which may leave the cilia still projecting or may include them. In this condition, by repeated transverse bipartition, large but very loosely coherent aggregates of individuals are formed, each within its own mucilaginous envelope.

Some such resting Palmella-like condition occurs as a phase in the life-history of all the *Chlamydomonas* species, but in this form it is the preponderating condition, and brings us very close to the types found in the Tetrasporaceae, which are all non-motile throughout their vegetative condition.

Dill, in 1895, published a most important paper which proved the constancy of the cytological specific characters for a large number of species of *Chlamydomonas*. At Kleb's suggestion Dill cultivated all the species he could collect, in Knop's solution of various strength, in water, in sugar solution, and on damp moss. He found that variations might thus be produced in the size of the individuals and in the thickness of their cell-walls, but that the cytological characteristics given above remain unchanged; with this solitary exception, that with *Chl. longistigma* nutritive salt solution causes elongation and frequently bipartition of the pyrenoids, so that four are present in place of the normal two.

Six of the species Dill cultivated were newly discovered by himself, which brought the number of well-established species up to fifteen, all of which can be recognized in their vegetative condition. This is a very remarkable result when we consider that with higher genera of Algae, *Spirogyra*, *Oedogonium* and other filamentous forms rich in species, it is only from the reproductive organs that the species can be certainly diagnosed.



This specific constancy in the most primitive type is in strong opposition to the idea of wide polymorphism brought forward by Hansgirg, Chodat, and Borzi, which associates different genera, and even members of different families, in the life-history of one individual.

Dill's paper contains further work of importance on the nature of the divisions by which the different species multiply vegetatively. We shall find, when discussing subsequently the relation of the Chlamydomonads to organisms of a decidedly animal character, that it is a marked characteristic of the division-process in the latter that this takes place in the antero-posterior direction, being generally a slow constriction starting at the two ends and proceeding to the centre.

In organisms of a decidedly vegetable character, division is a more rapid process, nearly simultaneous throughout the protoplast, and the *first* division nearly always takes place in a transverse plane.

In *Chlamydomonas*, Dill finds that, while the majority of species divides by a rapid transverse division, yet some divide by a first longitudinal division, not however of the nature of a slow constriction. The real interest of these two directions of division lies in this, that it is just those species which on evidence drawn from their sexual reproduction appear most primitive (i.e. least decidedly vegetal in character) that exhibit this more animal characteristic of a first longitudinal division. Between the species which divide first transversely and those which divide first longitudinally, Dill discovered a small interesting group of species in which a transitional condition occurs. In these forms the first sign of division is in the longitudinal direction, but the protoplast gradually rotates inside the cell-wall so that the division-plane separating the two sister-cells becomes transverse.

Still more striking than the variation in cytological characters found in the single genus *Chlamydomonas* is the variation in the sexual reproductive process. In every other genus of plants throughout the vegetable kingdom, however various the specific forms and biological characters, it is

found that the sexual reproductive process is in essentials constant and uniform for the genus, but here it is quite different.

*Chlamydomonas* affords an unique opportunity of acquiring a clear idea of the relation of the conceptions, genus, and species at the very foot of the phylogenetic tree, and we shall see that characters which are of ordinal and cohortal value very little higher up are here merely of specific value.

To illustrate this, let us consider the different types of sexual reproduction within this single genus. Isolated observations are found in the works of Ehrenberg ('38), Stein ('78), and others, but the first comparative study of the sexual process was made by Goroschankin ('91). In *Chl. Steinii* and the majority of species the contents of the vegetative cell divide by repeated bipartition into sixteen or thirty-two small planogametes. These are ciliated ovate bodies, and though much smaller than, yet of the same character as the vegetative body, except that they have no cell-wall. They escape by rupture of the mother-wall, and after swarming conjugate in pairs. As they are all of one size the union is isogamous, and a round zygote results, which surrounds itself with a thick wall and rests. This is a parallel procedure with that found in the higher *Protococcoideae*. In some other species the planogametes are provided with walls when first formed, and thus are quite like vegetative cells but for their size. This formation of walled planogametes is absolutely unknown in any higher plant than *Chlamydomonas*, and even in this genus is only found among the most primitive species, i. e. those which begin the vegetative division with a longitudinal bipartition. Before these planogametes actually conjugate they must get rid of their cell-walls, and for this process we find a continuous series of forms.

Thus with *Chl. longistigma* (Fig. 13) the walls of both the gametes about to unite are thrown off while the cells are still some distance apart; with *Chl. reticulata* (Fig. 13) one gamete seems to throw off its wall before the other, so that at conjugation only one quitted wall is to be seen; with

*Chl. Ehrenbergii* (Fig. 13) the walls are thrown off at the moment of union of the gametes, so that the two shells are found close to the resulting zygote; and finally, with *Chl. multifilis* fusion advances so far before the walls are actually discarded that the walls are left fused together at one part to form a single shell.

We have thus a series of forms, of which the last-mentioned is presumably the most primitive; from this we proceed up to gametes devoid of walls from their first formation.

The lower end of this remarkable series would be completed if we had a form in which the gametes conjugated by uniting inside their walls without throwing them off at all. Goroschankin ('90), in a separate and very complete paper, has shown that this is exactly what occurs in *Chl. Braunii* (= *Chl. pulvisculus*, Stein). In this species (Fig. 13), however, the walled gametes are of different sizes (heterogamous), and in their later stages non-motile by loss of their cilia, so that this form cannot take its place directly at the lower ends of an isogamous series. The smaller (male) gamete is about half the size of the female one; when they have swarmed close together the cilia are lost, and processes of the wall are put out at adjacent spots. These meet and fuse and form a canal, through which the contents of the smaller gamete pass over into the larger one. A wall is then formed in the canal, and the protoplasts of the two gametes fuse completely and form a zygote inside the wall of the female gamete. Occasionally several male gametes attach themselves to one female, but the contents of one only of them passes into the female. The similarity of this process with that occurring with the non-motile cells of *Spirogyra* and other conjugates is most remarkable, and it has been suggested that this is of real phylogenetic significance. At present we need only dwell on the variety of process found in this single genus from one extreme of the union of heterogamous walled aplanogametes to the other of the union of isogamous naked planogametes.

In addition to these forms Klebs ('96) has described a new species, *Chl. media* (Fig. 13), which shows a slight variation



from the type of *Chl. longistigma* in that the gametes before throwing off their walls begin to contract away from them at the posterior end and remain in this preliminary condition for some time, thus clearly marked as gametes. Klebs makes acute use of this visible character in his well-known experiments on the effects of conditions in inducing vegetative or sexual activity.

When a vegetative *Chlamydomonas*-cell is about to divide to form new individuals (the usual number in one mother-cell being four), it usually loses its cilia and becomes motionless, but this is not always so, and a mother-cell occasionally may be seen still moving after the four daughter-cells within it have acquired their new cilia. The movement is effected by the old cilia being still in continuity with the hinder end of the protoplast of the nearest daughter-cell. Such a condition (Fig. 13) is regular in one species of the closely allied genus, *Sphaerella Bütschlii*, see Blochmann ('85).

At some stage in their life-history all the species of *Chlamydomonas* are considered to pass into what is known as the Palmella-condition, where the cilia, eye-spot and contractile vacuoles may be lost and the resulting non-motile cells secrete mucilaginous walls within which they slowly divide to form irregular aggregates encircled by swollen traces of successive mother-cell-walls. Unfavourable conditions tend to produce this condition which was at first taken to be a separate genus. We have apparently in this condition the beginning of a vegetative existence in the narrower non-motile sense such as we find predominating in the Tetrasporaceae. At intervals in the life-history in this family the cells escape from their walls, put out cilia, and return to the motile condition as zoospores.

Formation of zoospores is then nothing but reversion to an ancestral type of vegetative existence for a biological advantage, and all the vegetative existence of the higher Algae is phylogenetically a new intercalation into the life-history of the motile Chlamydomonad which is permanently in the zoospore condition, though walled, and in which

zoospore-formation and vegetative cell-division are one and indistinguishably the same thing.

The cytological processes had been but little investigated till Dangeard ('99) contributed a long paper dealing with these in the Chlamydomonads and with the behaviour of the nucleus in division and in sexual fusion. This constituted a very distinct advance in our knowledge.

One of the points investigated is the relation between the protoplasm of the chloroplast and that of the rest of the cell in those types which are only just removed by evolution from colourless Flagellates. *Chlorogonium euchlorum* has the characters of a transitional form, and the earliest writers held the chlorophyll to be diffused through the cytoplasm generally, but Francé ('97) described differentiated discoid chloroplasts or a few spiral bands round the cell. Dangeard shows by skilful staining that there is but one chloroplast, as in the rest of the family, but that this is perforated with masses and strands of the central colourless protoplasm so that it may appear cut up into bands or disks.

*Chlorogonium euchlorum* seems to be very variable and erratic in its cell-characters, for the pyrenoids may vary from four to thirty-two, contractile vacuoles may be present in large numbers, and finally the individuals from one special habitat really seemed to have diffuse chlorophyll, colouring all the cell except a fragment at each end. The author is unable to suggest the cause and significance of these variations in one species of a family otherwise characterized by the constancy of its specific cytological characters.

The structure of the nucleus in the Chlamydomonads can be very clearly made out and all stages of karyokinetic division followed, and the chromosomes counted. The number for each species (10-30) is fairly constant.

Non-karyokinetic division of the nucleus was observed only in *Chlorogonium*, and this quite exceptionally. As this is generally held to signify degeneration, the author thinks it probably a prelude to death or sexual reproduction in this case.

A large part of the work deals with facts and theories as to protoplasmic and chloroplastic structure, among which there is signalized the discovery of a blepharoplast at the insertion of the cilia in *Chlorogonium*.

In studying the development of the zygote the author finds that the two chloroplasts fuse to form one, unlike their behaviour in *Spirogyra* (Chmielevsky, '90), and that sometimes two pyrenoids may actually fuse together though this is not the rule.

The paper concludes with a theory of sexuality, according to which the sexual reproduction in these primitive forms in which sexuality is only just evolved, is of the nature of a 'reciprocal autophagy.'

This theory regards gametes as in origin ordinary vegetative individuals which have for certain reasons 'run down' so to speak, and have acquired by natural selection sexual affinity which corresponds to a sort of hunger by which they are driven to fuse with, i. e. consume, some other individual by which they gain nutritive substance, and also energy perhaps of some special nature as well as the stored chemical energy. This enables existence to be carried on briskly again.

This way of looking at the matter fits many of the facts discovered by Klebs, such as that nutritive salt-solutions tend to avert sexual reproduction and bring on either parthenogenesis or a return to vegetative existence.

The theory also demands that in primitive sexuality the fusing nuclei shall have the same number of chromosomes as the vegetative individual, and this is found to be so among the Chlamydomonads. The necessary reduction probably takes place on the germination of the zygote.

Among more highly evolved plants the specializations to secure sexual reproduction mask the clearness of this interpretation, as when nuclear reduction becomes postponed in the sporophyte until the gametophyte is about to be produced. Many other phenomena of sexuality are brought into line with Dangeard's point of view in quite an interesting way.



We see from this section that though *Chlamydomonas* is the most primitive of Algae, yet it has an elaborate organization which should be accounted for by its descent; so in the next section we shall seek for light upon the evolutionary origin of this foundation-stone of the vegetable kingdom.

### SECTION III. *Flagellata*.

Until recent times investigators of the lower organisms strove to find a dividing line between those that ought to be classed as plants and those that must be regarded as animals. Attempts to base such a distinction on any single test-character resulted in groupings that were most obviously unnatural. The last and best of these characters in which hope was put, was the distinction between the methods of nutrition in the two kingdoms, but this also fails as a clue to a natural separation<sup>1</sup>.

The only scientific method of procedure is now recognized to be the inductive one. By this the organisms, such as they may be found to be, are grouped together by a consensus of their characteristics in small natural groups, and these again into larger aggregates. This, of course, in itself is by no means an easy matter, and until it had been approximately accomplished it could not be determined how far a line could be drawn between the two kingdoms without doing violence to these induced natural groupings.

Knowledge, however, gradually comes to hand, and it is certain that by no set characteristics can the distinction between plant and animal be pushed right down as a fundamental cleavage-line without separating otherwise closely allied genera and doing similar violence to natural groupings.

In the sea, of low forms of life (Protista) that intervene between the contrasted and undisputed plant and animal kingdoms, certain groups have been found by inductive investigations to stand out well characterized. The most striking of these, to the botanist at least, is that of the

<sup>1</sup> A few forms with well-developed chromoplasts, as *Chromulina*, ingest solid food.

Flagellata (sens. Klebs) and for this reason, that it contains just those organisms which approach closest to the lowest plants. Any battle to extend or restrict the territory of the plant must be fought out on the boundary to be drawn between the Algae and the Flagellata.

It is thus impossible to treat critically the lower green Algae without giving some attention to the groups of Flagellata which adjoin them. That we are able to consider these in any satisfactory way is due almost entirely to Klebs, and the masterly quality and immense quantity of his work make a deep impression on any one who goes into the subject. In 1893 Klebs published his elaborate study of the organisms of this group; and this account serves as a starting-point for all future work and speculation. In these 'Flagellatenstudien,' Klebs maintains that this name corresponds to a natural group of organisms having certain characters in common. The critical characters are those relating (1) to structural organization, (2) to the manner of vegetative division, and (3) to the nature of the resting stages.

1. Broadly speaking the Flagellates have the same type of organization as the lowest Algae—the Chlamydomonads—namely, a motile, more or less oval, solid protoplasmic body with a central nucleus and a specialized anterior end with two or more cilia and contractile vacuoles. The cell may be colourless or have green or brown chromatophores.

In the peripheral envelope, however, we find a difference. Outside the protoplasm there may be a dead mucilaginous envelope, but there is no cell-wall as in the lower Algae. The protoplasm may be limited by a very thin specialized layer (periplast) or by a thick special layer (plasma-membrane) as in *Euglena*, but these are integral parts of the cell and divide with it, unlike a true cell-wall. Further, starch is not found, chloroplasts are usually discoid, and the nature of the wall permits amoeboid movements to occur.

2. The division usually takes place in the motile stage, and is always longitudinal and of the nature of a slow constriction into two halves starting from the fore end.

3. The resting cells are cysts formed vegetatively, and zygotes do not occur, as no sexual distinction or reproduction occurs in the Flagellata.

Five subdivisions of the Flagellata are proposed by Klebs.

I. *Protomastigina*, including the small simplest forms which have only a periplast, are abundantly amoeboid, and always colourless. These absorb food either by their whole periphery or only at the fore end, where however there is no definite mouth. (*Bodo*, Fig. 13.)

II. *Polymastigina*, larger colourless forms of rather similar organization and mostly taking up solid food at definite places with a mouth, but this never at the fore end. This group has no plant affinities at all. (*Hexamitus*, Fig. 13.)

III. *Euglenoidina*, large forms with well-developed plasma-membrane, 'metabolic' but never amoeboid. At the fore end is a mouth through which the cilia are attached to the wall of a vacuole (Wager, '00). The body is colourless or with green disk chromatophores. The nutrition may be holophytic, saprophytic, or animal. (*Euglena*, Fig. 13.)

IV. *Chloromonadina*, body somewhat amoeboid and without plasma-membrane, numerous discoid (yellow-green) chloroplasts. The nutrition is holophytic and division takes place in a mucilage-invested resting stage. Only two or three genera of this type are known. (*Vacuolaria*, Fig. 13.)

V. *Chromomonadina*, cells single or in colonies; structure various, no plasma-membrane. Most of the numerous forms have one or two brown chromatophores and the nutrition is holophytic.

The last three groups with coloured chromoplasts suggest affinities with Algae, and Klebs in 1893 suggested an undefined connexion between the two green groups and the Chlamydomonads. The affinity between the Chromomonadina and certain groups of brown Algae does not admit of much doubt, and the nature of this affinity, on which recent work has thrown new light, will be considered in Section V.

The Euglenoidina are a rather specialized group which does not obviously lead to the Chlamydomonad type. The



Chloromonads are nearer the latter but the cell-type with many yellowish-green chloroplasts is different, and recently a definite affinity has been suggested between these Chloromonads and a special group of green Algae (cf. Sect. IV). It seems to me then most reasonable by exclusion to derive the Chlamydomonads directly from the Protomastigina. As transitional forms we have *Pyramidomonas*, and *Polyblepharis* which Wille places in the Chlamydomonadaceae, but which though little known seem to be somewhat more primitive than *Chlamydomonas* and almost devoid of a cell-wall. Possibly they have been derived from green forms among the Chromomonads, but these suggestions must suffice here.

It appears that no organisms of preponderating plant-characters have been evolved from the *Euglena* type. Its bright green colour and its power of holophytic nutrition superficially suggest an algal nature, and these with its well-developed plasmatic membrane and its paramylum-grains, which though not starch are an allied substance, seem to show that it has evolved from the Protomastigina in the plant direction, so to speak, or rather in a parallel direction. Still, by a consensus of the critical characters, it is a Flagellate beyond question. Closely allied to *Euglena* are some colourless forms, and Zumstein ('99) has just shown that *Euglena gracilis* itself may exist as a colourless saprophytic form. He finds that if a pure culture of *Euglena gracilis* be placed in a sugar-solution in the dark it multiplies abundantly and may become quite free from chlorophyll. When the organisms are replaced in pure water in the light, the culture develops chlorophyll and becomes green again and assimilates carbon dioxide. Thus either a saprophytic or holophytic nutrition can be maintained. Grown in the light in a medium rich in nutritive matter *Euglena gracilis* is, according to the author, colourless, which suggests that it prefers a saprophytic nutrition and only develops chlorophyll in order to obtain carbon by assimilation of carbon dioxide when other sources are scanty. Here we have the spectacle of a single species lightly passing backwards and forwards over the supposed dividing line.

between two opposed methods of nutrition, the algal and the fungal, just as it may happen to be well fed or not.

Bohlin ('97a) has described a similar case of alternative nutrition for *Chloramoeba* which belongs to a different class of Flagellates, so that in the hypothetical transition from the colourless Protomastigina to the Chlamydomonads, the necessity for developing chlorophyll is not a serious difficulty, in the light of these phenomena.

Just as green organisms occur among the Flagellata so colourless forms are found among the lower Algae. There should be really no more objection to letting such colourless forms take their place in the respective families to which a consensus of their characteristics entitles them, than is made as to the position of the chlorophyll-free Flowering Plants.

*Chlamydomonas* possesses one species, *Chl. hyalina*, which is, apparently, characteristically colourless and saprophytic. Further, related to *Chlamydomonas*, there exists a small group of colourless organisms of which *Polytoma uvella* is the best known. These have been carefully monographed by Francé ('94). They have the cell-structure of the Chlamydomonad type and starch is found in the cells, but they have a thick or thin out-standing wall. They divide by transverse or oblique divisions into groups of four or eight daughter-cells, usually while in the motile stage. Conjugation of gametes (indistinguishable from vegetative cells) has been observed. This group has thus all the critical characters of the Chlamydomonads except the green colour. Early observers of course classed them as animals. Dangeard ('88) proved that their nutrition was saprophytic and that their cell-wall prevented any ingestion of solid particles. It seems clear that they form a little family of which all the forms are saprophytic, and that they have probably evolved from some one green organism of the Chlamydomonad type. This necessitates their being placed as a sub-family in the Volvocaceae. They show certain resemblances to a special group of colourless Flagellates, and it is a theoretical possibility that a saprophytic organism of preponderating plant-characters might be evolved from a

colourless Flagellate without any green forms being involved at all.

One other interesting organism of this nature (*Sycamina*) has been described by Van Tieghem ('80). This seems to be a colony of the complexity of *Volvox*, but, though blackish, quite devoid of chlorophyll. Its methods of reproduction are unknown, so that it can only be doubtfully suggested that it must stand to the Volvocineae in some such relation as *Polytoma* does to the Chlamydomonadeae.

All these colourless forms were excluded by Wille from his Volvocaceae, but further investigation of their characters and nutrition necessitates their inclusion in the group. See p. 649.

From this outline of the possible Flagellate origins of the primitive Chlorophyceae we may pass in the next section to consider a special case, in which it has been proposed to create an independent phylum of green Algae and to derive it from the Chloromonadales.

#### SECTION IV. *Heterokontae*.

Borzi ('89) proposed to group together into one special cohort—Confervales Bz.—certain forms of the Chlorophyceae which had been previously distributed among various groups. This idea he elaborated in his well-known *Studi Algologici* ('95), and also placed in the group several new genera discovered by himself. The characteristics of this cohort are essentially cytological. All cells contain discoid yellowish-green chloroplasts which are usually numerous and which lack pyrenoids. The stored-up product of assimilation is not starch but some fatty substance. The zoospores have also discoid chromatophores, typically two lateral ones, and generally only one cilium.

The members of this cohort are unicellular, filamentous or coenocytic, and so, as with the Chlorophyceae in general, the included forms are an ascending series from the structural point of view. The lower ranks of this new cohort include the Sciadiaceae, which are practically the Characieae of Wille, consisting mostly of unicellular forms attached by the base,



as *Ophiocytium* (including *Sciadium*), *Peroniella*, *Characiopsis*, Bz., *Chlorothecium*, Bz., and *Mischococcus* which previously had been in the Tetrasporaceae. The genus *Conferva* is also included here, and on the above characteristics is separated widely from *Microspora* which has none of them, but with which it had always been closely associated. The higher ranks of this cohort include three rather divergent types, *Bumilleria*, *Botrydiopsis* and *Botrydium*.

In 1897 Bohlin published an interesting paper containing a detailed study of the cytological characters of this group.

With regard to the structure of their cell-walls it had long been known that filaments of *Conferva* and *Microspora* break up across the middle of the cells into short cylindrical units consisting of the halves of two adjacent cells with a septum separating them. These are generally known as H-pieces from their appearance in optical section. Those of *Microspora* are homogeneous and of pure cellulose, but in *Conferva* (Fig. 14, *a*) they consist chiefly of pectic acid derivatives, and on swelling with potash show a special layered structure. On investigating filaments in all stages of division the growth of the cell-wall revealed a new type. The unit of growth consists of the halves of two adjacent cells, and thus the two halves of any given cell are of different age. On transverse division the new septum with the new ring-thickening at its periphery constitutes the starting-point for a new H-unit, and while it thickens by obvious apposition layers, these also extend and elongate the cells on either side of the septum and so push two older units apart. In the diagram of the swollen wall structure in Fig. 14, *b* is a young cell and *c* an old one. Considerable interest attaches to this procedure in itself, but this is much increased on its being shown that *Ophiocytium*, which Borzi had brought from a far-off family to join *Conferva*, possesses an interesting variation of this very type of growth. *Ophiocytium* when mature is a long curled cylindrical plurinucleate single cell which breaks across just near the top to liberate spores. This top cap is found to be the unaugmented half of the originally very short cell.

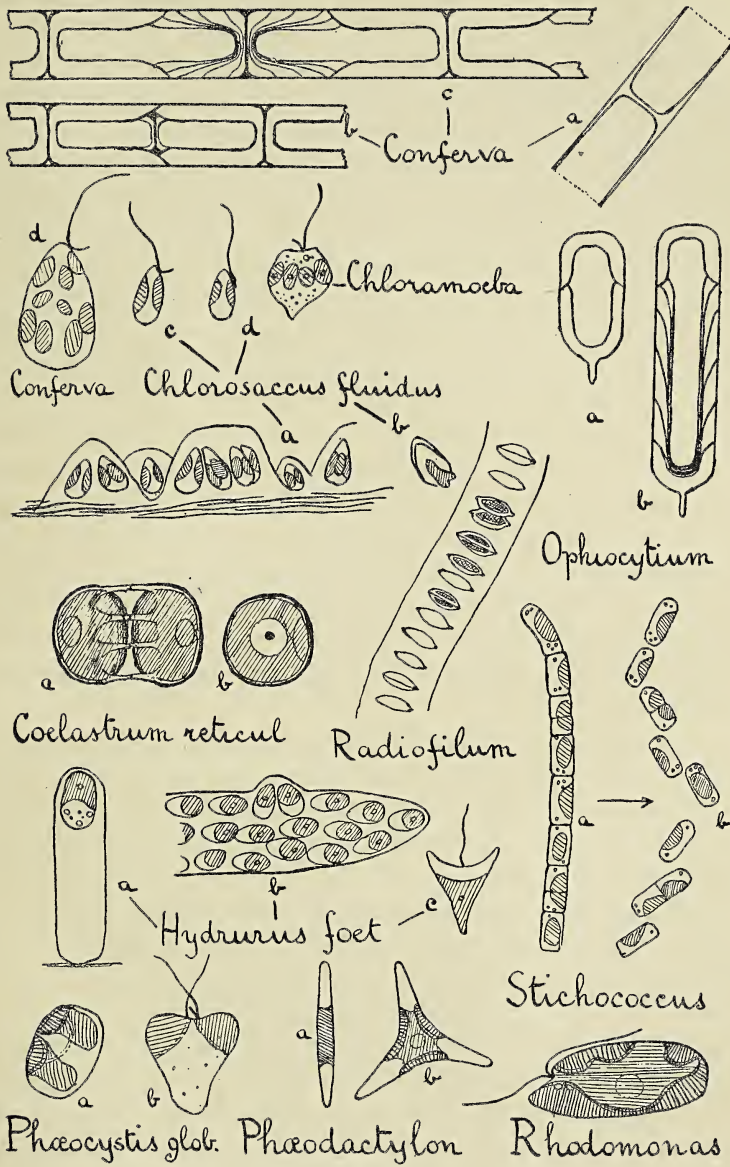


Fig. 14.

The whole of the elongation of the growing cell has been produced by the apposition of layers inside the lower half with cotemporaneous extension and elongation of them so that the lower half of the cell behaves just like half a growth unit of the *Conferva* type (cf. the schema in Fig. 14; *a* is the young cell and *b* a half-grown cell). The two genera are thus closely related, but one is unicellular and the other pluricellular, so that we have here another case against primary groups based on these characters.

The yellow-green chromatophores of this group give a blue odour with strong hydrochloric acid due to the abundance of xanthophyll, while typical Chlorophyceae become yellow when so treated. In bright light, fine refractive granules are first produced in the cell, and as assimilation goes on these run together to form large oil-globules, soluble in chloroform but not in alcohol. A small amount of hexose can be extracted from the cell by water, and it is probably the earliest product of assimilation and reduced afterwards to fat.

*Vaucheria* seems to be the only green Alga outside the Confervales which has chlorophyll possessing these characters. What phylogenetic significance this may have is at present doubtful.

The very close relation of *Ophiocytium* with *Conferva* leads Bohlin to rearrange the genera within the Confervales into three families.

I. *Confervaceae*, thallus uni- or multicellular, gametes biciliate, no pyrenoids.

*Conferva*, *Ophiocytium*, *Bumilleria*, *Botrydiopsis*.

II. *Chlorotheciaceae*, cells single or a thallus, gametes uniciliate, no pyrenoids.

*Mischococcus*, *Peroniella*, *Characiopsis*, &c.

III. *Botrydiaceae*, thallus unicellular showing root and shoot, multinucleate, pyrenoids present in young plant. *Botrydium*.

Among the Flagellata there is a form which has some of the Confervales characteristics, namely a number of discoid, yellow-green chloroplasts without pyrenoids. This is the genus *Vacuolaria* (Cienkowski, '67) (Fig. 14) of the Chloro-





ERRATUM.

Vol. XIV, p. 674, line 11, *for* odour *read* colour

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monadina. Not much was known about it, and as a simple mouthless green form Klebs had suggested that it might be a link between the colourless Flagellata and the Chlamydomonads. None of the Chlamydomonads, however, have these cytological characters, so that this type seems to complicate the matter rather than simplify them.

In 1897 Lagerheim discovered a similar but new genus, *Chloramoeba* (Fig. 14), the life-history of which was adequately investigated by his pupil Bohlin ('97). This has a roundish body, no wall or plasmatic membrane, a contractile vacuole at the fore end, a central nucleus, 2-6 discoid chloroplasts of a yellow-green colour, and two cilia, one twice as long as the body and the other quite short and often curled back against the body so as to be invisible.

The body exhibits marked amoeboid movement. The chloroplasts give a blue colour when treated with strong hydrochloric acid, as do those of the Confervales group, and the assimilation product, as in that group, is an oil, insoluble in alcohol.

Resting cells may arise by the encystment of solitary motile cells, but no Palmella-stage. This organism then is a typical Flagellate, while *Vacuolaria*, which divides in a palmelloid mucilaginous resting condition, exhibits somewhat more vegetal characters (Fig. 14).

Further, while *Vacuolaria* is always green, *Chloramoeba* can adapt itself to a saprophytic nutrition in the dark, like *Euglena*, and become quite colourless. On transferring the organism to the light, in water free from organic material the yellow-green colour is regained, as has been already mentioned.

The group of Chloromonadina is then well established with the primitive form *Chloramoeba* and the more vegetal form *Vacuolaria*, and the points of similarity in cell structure with the Confervales are striking and suggestive of some affinity, if primary importance is to be attached to cytological characters.

Within a year a paper of great interest was published by Luther ('98), in which he described a new organism, *Chloro-*



*saccus*, which seems beyond doubt to connect up these green Flagellates in a direct line with the Confervales. *Chlorosaccus* (Fig. 14, *a*) has a superficial resemblance to *Tetraspora*, but is of a yellow-green colour and grows submerged, and attached to water-plants, forming irregular hollow mucilaginous spheres.

The individual cells in the mucilage are pear-shaped, with the smaller end outwards, and when division is rapid they are found grouped in fours, but this arrangement is soon obscured.

The chromatophores are parietal disks, several in number, and they give the blue colour with hydrochloric acid. There are no pyrenoids. When the cells are alive there appears to be no cell-wall within the mucilage, but after death a shell of wall can be made out which is thick at the superficial small end and very thin at the big central end, or even incomplete so that it may spring off the dead protoplast (Fig. 14, *b*)<sup>1</sup>. This structure is of great interest as a transition between the unwallled Flagellate type and the walled vegetal type, and its greater density at the exposed end agrees with *à priori* considerations. Staining shows that the wall is of a pectic nature, as in *Conferva*, and no cellulose reactions can be detected.

The cell-division takes place parallel to the long axis of the individual cells which is a Flagellate character, and four daughter-cells arise nearly simultaneously. Individual cells may enlarge to form resting akinetes with thick walls, and these drop out of the colony.

The greater part of the existence is thus non-motile, but zoospore-formation takes place under suitable conditions. The zoospores, at first pear-shaped, escape through the mucilage, and when free their naked mass contracts to a rounder form. Each has two lateral discoid yellow-green chloroplasts, and doubtfully an eye-spot. At the fore end are two cilia, one three times the body-length, projecting forwards, and the other less than the body-length, thin and bent. If the zoospore be killed with iodine (Fig. 14, *c*), both

<sup>1</sup> *Pilidiocystis*, Bohlin ('97 *c*) has a somewhat similar wall.

cilia are clearly seen, but if killed with osmic acid (Fig. 14, *d*), the short one curls back tightly against the body, and is invisible. After a few hours' swarming the zoospores come to rest, secrete mucilage round them, and begin dividing to form a new colony. There is no sexual reproduction.

The relation of *Chlorosaccus* to *Chloramoeba* seems clear. Its *Tetraspora*-like aggregation and the shortness of the motile stage are plant characteristics, while its incomplete wall and absence of sexual reproduction are Flagellate characteristics. Luther, attaching much importance to the vegetative division being parallel to the long axis of the cell, allows this to weigh down the balance and classes it as a Flagellate in the same family with *Vacuolaria*; but it seems to me that he overrates the importance of this, since Dill has shown that the lower Chlamydomonads divide in this way, and as division must go so if the colony-membrane is to expand tangentially without cell-displacement. Perhaps then it may be ranked as an Alga rather than a colonial Flagellate. At all events it is very nearly balanced on the line between the two classes, and only serves to illustrate the absence of absolute cleavage-lines and how in a difficult case one's decision may almost be called 'subjective.'

The great stumbling-block in the way of deriving the Confervales from a *Chlorosaccus* type, just as the Confervoideae are derived through a *Tetraspora* type, was obviously the difference in the ciliation of the zoospores.

The zoospores of the typical genus *Conferva* have several discoid yellow-green chloroplasts, as analogy would indicate, but they are described by Klebs and other good observers as having only one cilium—a long one. Luther made the important discovery that they really have a second short cilium, and if they have been killed with iodine this can be seen standing out from the body (Fig. 14, *d*). Most fixing reagents, however, cause this one to curl back against the body and to become invisible. He found also that just the same holds good for *Botrydiopsis*, another genus of the Confervales which he investigated. This character then appears,

with the other cytological characters, to hold right through from the typical Flagellate *Chloramoeba* to these high filamentous Algae.

Luther proposes to regard these forms as evolved from the Chloromonadina of the Flagellates and to take them therefore right out of the true Chlorophyceae which are regarded as evolved from the Protomastigina group of the Flagellates, and to make a separate main group of them equivalent with that of Chlorophyceae. For this he proposes the name of Heterokontae, and divides it up into a Flagellate group *Chloromonadales* with the ascending series of forms:—*Chloramoeba*, Bohl., *Vacuolaria*, Cienk., *Chlorosaccus*, Luther, and an algal series *Confervales*, Bz.; containing the families Confervaceae, Chlorotheciaceae and Botrydiaceae.

The evidence for this separation seems adequate, and it is an immense advance in the direction of a natural phylogenetic classification of the green Algae. Possibly other series of forms may also be removed from the Chlorophyceae and have an independent evolution attributed to them, thus rendering the Algae still more polyphyletic.

The relation between the green Algae and the Flagellates may be briefly summarized thus. The green Euglenoidina give rise to no algal forms, the green Chloromonadina have given rise to the Heterokontae series of green Algae, while the colourless Protomastigina have probably produced the Chlamydomonad type and all that has arisen from it.

In conclusion I propose to give a short account of the relation between the brown Flagellates and some of the newly described forms of the Phaeophyceae.

#### SECTION V. *Primitive Phaeophyceae.*

Not many years ago (see Kjellman ('91) in Engler and Prantl) the simplest structural forms recognized amongst the Phaeophyceae were elaborately branched filaments such as occur in the marine *Ectocarpus* and the fresh-water *Pleurocladia*; Klebahn ('95), Wille ('95).



In the absence of any primitive forms any theory of the origin of this group was then simply a matter of speculation, but a possible derivation from brown Flagellate organisms was suggested as a parallel with the origin of the green Algae. Links to substantiate such a line have now been discovered, and this theory may be considered as at the present time firmly established. It is of the greatest interest to note that the intermediate organisms show a striking parallelism in grade with those in a similar position on the phylum of the Chlorophyceae.

We have thus a rising series :—

1. Organisms which are generally admitted to be Flagellata, as *Synura*, *Uroglena*, and *Hydrurus*.

2. *Phaeocystis*, which approaches the border line of the Algae.

3. *Phaeococcus* and *Entodesmis*, which correspond to the Tetrasporaceae among the green Algae.

4. *Phaeodactylon* and *Stichogloea*, parallel with the Pleurococcaceae.

5. *Phaeothamnion*, corresponding to a very simple type of Confervoideae and leading to—

6. The typical Phaeosporeae which have the unilateral swarm-spore and so-called uni- and multilocular sporangia.

At the present time most of these intermediate groups are very imperfectly known, but taken together they seem to form a bridge over the gap, which is sufficiently strong to carry this hypothesis of the evolution of the Phaeophyceae from Flagellata quite independently of the evolution of the Chlorophyceae and the Heterokontae.

We will go briefly through some of the points of interest in this series of forms.

It is a general character of this phylum that the chromatophores are brown, and the product of assimilation neither starch nor oil, and that the cilia of the motile cells are usually unsymmetrical.

The first group of these forms belongs to the sub-family Chrysomonadina of the Chromomonadina. Here we find

many unicellular naked brown Flagellates and some well defined colonial forms, of which we may mention three.

*Synura* (Klebs ('93), Schmidle ('99)) consists of a motile colony of about ten heart-shaped brown cells, each biciliate and grouped as in *Pandorina*, with an investing mucilage through which the cilia protrude. The cells are all naked and the colony is not a true coenobium, as it grows to mature size by division of its cells from a single free motile cell.

*Uroglena* (Klebs ('92), Iwanoff ('99)). The cells are of the same type, but the colony resembles *Volvox* in having a large hollow central space full of mucilage and a number of ciliate cells round the periphery; but the colony may divide into two by constriction. On the phylum of brown organisms these colonial forms which resemble the Volvocaceous types of aggregation are found to be made up of true Flagellate cells, i. e. naked and dividing longitudinally, instead of true algal cells as on the green phylum. In spite of this, Bütschli ('86) grouped these two families together in his Phytomastigoda.

*Hydrurus* (Fig. 14, *a, b, c*), long thought to be a green Alga, was by Klebs ('93) shown to consist of a number of unciliated cells each with one phaeoplast and devoid of a cell-wall, but all imbedded in a branched cylindrical mucilaginous matrix which may be 30 cms. long and yet branches with a regularity rarely met with among the Algae<sup>1</sup>. The cells are single at the apices of the branches and form a peripheral layer lower down (*b*). The morphological differentiation of the branch-system found here, is unparalleled in such a loose aggregation of apparently unconnected cells: thus, the opposition of base and apex is very marked and the basal cells never give rise to branches; the centrally situated cells seem to have lost the power of division, and growth in length is entirely conducted by the single apical cells, the branching being usually monopodial.

Thus while the whole is clearly a colony of unicellular units, it has yet attained the morphological correlations of intimate multicellularity. The apical cells characteristically divide

<sup>1</sup> Phaeodermatium is generally held to be an unbranched cushion-like form of a *Hydrurus* colony.

longitudinally, and one half becomes the new apical cell while the other is displaced below it.

For reproduction the cells become free and swarm as tetrahedral zoospores with a colourless ciliate apex (*c*). These subsequently come to rest, attach themselves by the clear apex and secrete a long mucilaginous column so as to push themselves up on a wide stalk (*a*). The base of the cell is thus directed to the apex of the stalk, and this relation is maintained all through the branching system. Lateral branches arise acropetally by peripheral cells, at intervals, taking on apical growth. Of course there is no sexual reproduction. The cells contain no pyrenoids and the product of assimilation is a highly refracting liquid, the so-called 'leucosin.' The essential point which distinguishes *Hydrurus* from other colonial Flagellates is that the whole organism is *non-motile in the main part of its existence*. It only reverts to its ancestral motility at the swarm-spore stage, and this of course for biological advantage in spreading the species. Now this non-motility is characteristic of the plant-type, and those who attach *absolute* importance to this consider that *Hydrurus* has evolved over the border-line and must be considered to be an Alga, though it is clearly on a little branch-phyllum of its own and not on the main Phaeophyceae-phyllum. Its remarkable morphological differentiation supports this. If, however, motility is to be the absolute test it is clear that conversely all the Volvocaceae must be regarded as Flagellates; Bütschli, Scherffel ('00). Adopting a *consensus* of characteristics as our standard, we may keep the Volvocaceae as plants and yet hesitate to call *Hydrurus* one.

*Phaeocystis*. Lagerheim ('96), Scherffel ('98), ('00). The two known species of this genus form mucilaginous spherical colonies visible to the naked eye and floating motionless in the sea. The centre of the colony consists of thin mucilage, and the naked round cells with their two phaeoplasts are sparsely scattered over the denser periphery of this very fragile organism. Contractile vacuoles, eye-spots and pyrenoids are absent, and leucosin is the product of assimilation. *Phaeocystis* was first



described as a species of *Tetraspora*, which it superficially resembles, and it reproduces by zoospores.

The individual cells are non-motile, and the mucilage may serve to float them by entangling air, or to protect them from small larvae. In Fig. 14, *a*, a vegetative cell with two band-phaeoplasts and a globule of leucosin is shown, and in *b* a zoospore which has the very unusual equipment of three cilia, two long and one short, and also has its chromatophores at the anterior end.

Here again the essential non-motility is held by Scherffel to put this among the Algae, but Lagerheim and most authorities place it among the Flagellata on account of its naked cells and probable longitudinal division. The absence of cilia, eye-spot and vacuoles, however, seems to me to indicate a long established abandonment of the Flagellate type. The characters which Klebs so successfully drew up to differentiate between Flagellates and green Algae are not equally decisive between Flagellates and brown Algae, which indeed is only natural as they are independent evolutions. All speculators have so far tried to use the same sets of characters on both phyla. I would suggest that this should be abandoned, and that it should be recognized that on the brown phylum the evolution of colonial aggregates of increasing solidarity has gone ahead of the evolution of a type of individual cell which is definitely algal, compared with the relation of these two characters on the green phylum. Thus the majority of unicellular motile *green* organisms are Algae, while the majority of *brown* are Flagellata. Organisms of the *Volvox*, *Pandorina*, and *Tetraspora* type of aggregation are found to be algal (in cell-type) if green, and Flagellate when brown. This seems tenable and removes the difficulties of a rigid system, but shows how unreal and unimportant is the separation of the kingdoms. Rising higher on the phylum we come to organisms which have sufficient plant-characteristics to leave no room for discussion.

*Phaeococcus* (Borzi ('93)) consists of spherical cells walled and non-motile, of which four are imbedded in a gelatinous matrix

(recalling *Gloeocystis*). They divide in all three directions and possess zoospores with unequal laterally inserted cilia. The conjugation of planogametes to form a zygote has been observed.

*Entodesmis* (Borzi ('92)) consists of elliptical, walled cells in tabulate mucilaginous colonies. The cells form zoospores with two unequal lateral cilia. These two genera have no Flagellate characters and are clearly of the status of the Tetrasporaceae.

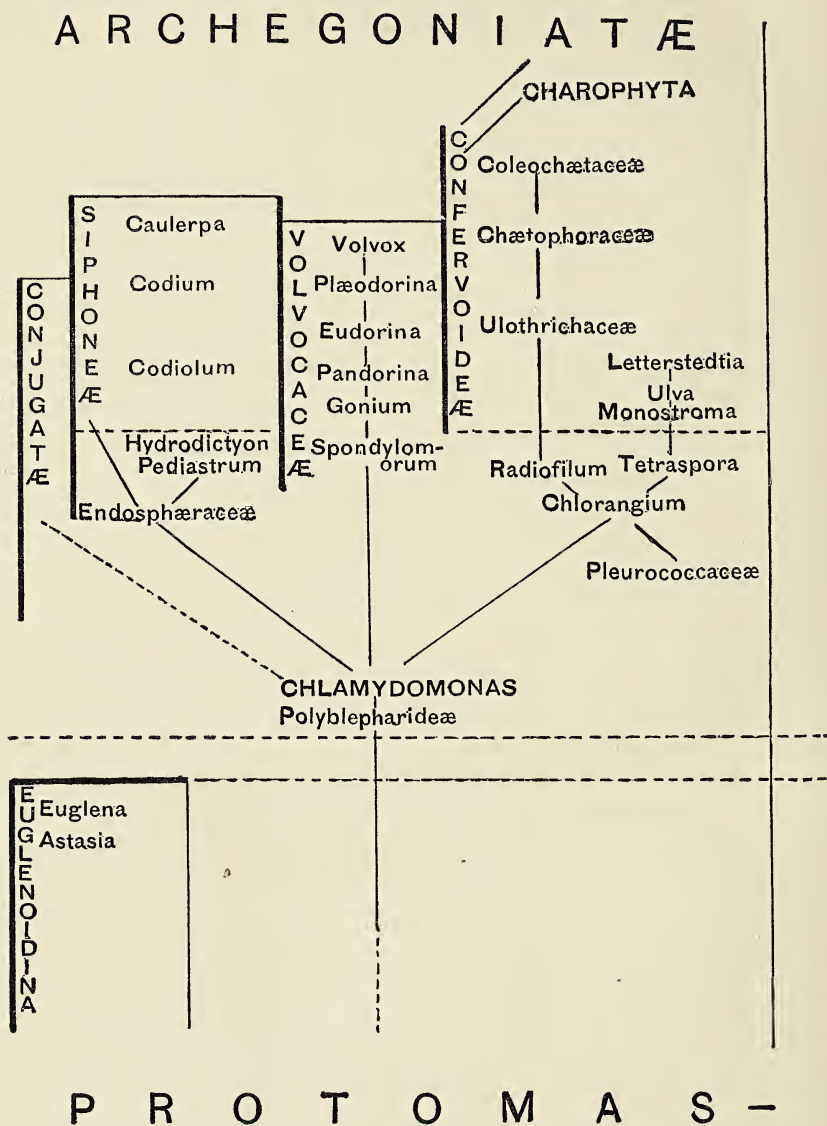
*Phaeodactylon* (Bohlin ('97)) has a star-like cell with three arms in one plane and a central brown chromatophore. The cell-wall cannot be resolved into two pieces as with Diatoms. It floats freely in pools of brackish water and is considered to multiply only by vegetative cell-division. In Fig. 14, *a* and *b* represent aspects at right angles to each other.

*Stichogloea* (Chodat ('97*a*)) also multiplies only by vegetative division, and thus with the previous genus represents the Pleurococcaceae-type. The cells are ellipsoid and grouped in fours to form a mucilaginous colony.

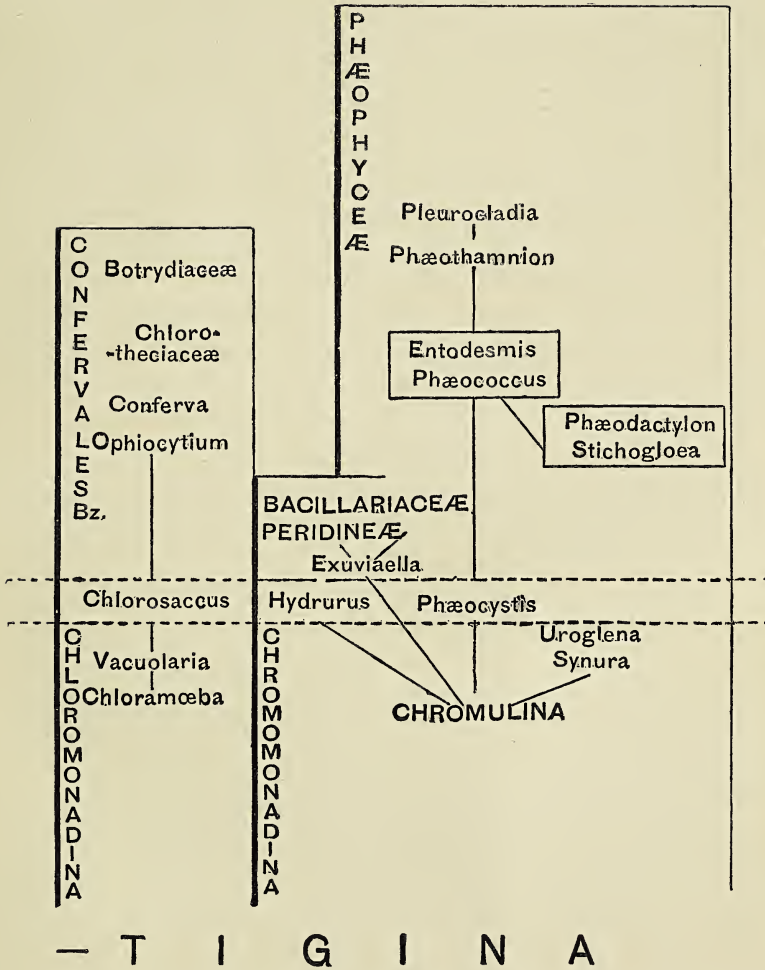
*Phaeothamnion* (Lagerheim ('84)) brings us to the filamentous brown Algae (corresponding with the green Confervoideae). It forms small tufts of slightly branched filaments growing attached in fresh water. Its discoverer, after some hesitation, placed it, in spite of its colour, in the green Confervoideae because he found its zoospores to have two equal apical cilia. This is accepted by Wille ('90) in Engler and Prantl, but Borzi ('92) asserts that the zoospores have two lateral unequal cilia. Our new knowledge of primitive brown forms shows us that this character is not an absolute criterion. On general grounds later authorities place it on the brown phylum. *Phaeothamnion* also forms 'palmella' states.

*Pleurocladia* (Klebahn ('95), Wille ('95)) is a larger branched fresh-water brown Alga, and may be regarded as the simplest of those conforming to the long-recognized type with zoospores and gametes; and here we may leave this ascending series of brown forms on well-established ground.

While all the higher brown Algae are marine, it is perhaps







significant of the Flagellate origin that nearly all of the primitive forms inhabit fresh water.

The gaps in the series that we have gone through are too large to allow very precise phylogenetic speculation, but the schema on pp. 684, 685 embodies the views of Scherffel, Lagerheim and Bohlin.

The origin of the third great group of Algae, the Rhodophyceae, is still a vexed question which is discussed at some length by Schmitz; see Hauptfleisch ('94). Until recently no Flagellate with the characteristic Floridean red colour had been described, so that such an origin, parallel with that of the Chlorophyceae and Phaeophyceae, seemed out of the question.

Karsten ('98) figures such a marine organism. *Rhodomonas* (Fig. 14) with two cilia and a large single indented chromophore of true Floridean red colour. Its life-history is at present unknown. The occurrence of a typical Flagellate with this pigment just suggests the possibility of such an origin of the Bangiales and Florideae, but no more than this can be said.

#### POSTSCRIPT.

Since these sections on the lower Algae and the Flagellata were written there has appeared a new treatise on the Flagellata by Senn ('00), who has worked under Klebs. This comes as an addition to the original scheme of Engler and Prantl's 'Pflanzenfamilien,' and clearly enforces one of the main arguments of this paper, viz. that without studying this group, though it is admitted not to be a plant-group, a clear comprehension of the relations of the primitive Algae cannot be obtained.

The arrangement in this excellent work follows that proposed by Klebs ('92) for the pigmented forms, but some further useful subdivisions are carried out for the colourless forms furthest removed from the vegetal type.

Senn hesitates to accept the suggested relation of the Conserveales with the Flagellate *Vacuolaria* on the grounds that the latter is too highly differentiated, but the simple hypothesis of a common ancestor destroys none of the interest of the theory.

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## Recent Work on the Results of Fertilization in Angiosperms.

BY

ETHEL SARGANT.

THE main conclusions drawn by Nawaschin (6) from his recent researches on the fertilization of *Lilium Martagon* and *Fritillaria tenella* were published in Russia more than two years ago. They were first brought to the notice of most European botanists by a paragraph in the Botanisches Centralblatt of January 4, 1899 (6), and this was shortly followed by the publication of similar results by Guignard (3). These results, obtained by independent observations on material almost identical, formed the most complete and striking confirmation of Nawaschin's work. Both botanists had examined the embryo-sac of *Lilium Martagon*—the classic ground for such observations—and both had come to the startling conclusion, with which we are now familiar, that the pollen-tube sets two generative nuclei free in the embryo-sac, one of which fuses with the nucleus of the egg-cell, the other with that which we are accustomed to call the definitive nucleus of the embryo-sac. This definitive nucleus is itself a compound structure, formed by the fusion of the polar nuclei. In *Lilium Martagon* the generative nucleus sometimes reaches the polar nuclei before they have met, sometimes afterwards, but in either case the nuclear fusion in the

[Annals of Botany, Vol. XIV. No. LVI. December, 1900.]

centre of the embryo-sac is clearly seen to be a fusion of three nuclei, not two.

The same phenomena were observed by Nawaschin in *Fritillaria tenella* and by Guignard in other species of *Lilium* (*L. pyrenaicum*, &c.). Botanists were therefore presented almost simultaneously with two independent sets of concordant observations made by two observers of the first rank on a few closely allied species. So unlooked-for were their results, that evidence less irresistible than this might well have needed confirmation. As it was, the facts were generally accepted at once (Strasburger, 10), and became the starting-point for further research. This has now proceeded for over a year in more than one direction.

As facts have accumulated, the question of their theoretical importance has become more pressing, and we have now several conflicting opinions on the subject. I propose in this paper to collect together, so far as is possible, all the work which has followed the original discovery, and to consider its bearing on the theoretical considerations involved. Such an attempt in these early days can have at the best no permanent value. But it is sometimes useful in the early stages of an investigation to take stock, as it were, of our assets of knowledge. This is more particularly the case when the subject is of general interest, and the work done in it is scattered through a number of memoirs by specialists. Much of this work has already been collected by Strasburger (11) in his recent important paper in the *Botanische Zeitung*, to which I shall have occasion to refer again.

Four distinct questions were suggested by the discovery of 'double fertilization'<sup>1</sup>. They may be briefly stated as follows:—

1. Is the phenomenon of double fertilization general among Angiosperms, or to be considered as peculiar to a few species?
2. Does the twisted (vermiform) shape of both generative nuclei in *Lilium* indicate that they have an independent power

<sup>1</sup> The expression 'double fertilization' is used for convenience throughout this paper, and is not intended to imply any view as to the nature of the conjugation.



of motion within the embryo-sac, and if so, can they be considered as much reduced spermatozoids?

3. What light is thrown by these researches on the homology of the structures contained in the typical embryo-sac of Angiosperms?

4. Can the fusion of the second generative nucleus with the definitive nucleus of the embryo-sac be considered as a true act of fertilization?

It will be convenient to deal with these questions separately, and to consider the work of the past two years with reference to each in turn, rather than to adopt a strictly chronological arrangement. I shall also mention in connexion with each any work of an earlier date which seems to me to bear on the point, or to suggest an opening for further research.

#### I. OCCURRENCE OF DOUBLE FERTILIZATION IN ANGIOSPERMS.

Considering that we are dealing with the work published during little more than a year, and that the labour involved in such research is great, the number of species in which double fertilization has been observed since the original discovery may be called large. The following is a list, as complete as I can make it, up to the present date (Nov. 14, 1900). It includes the original observations on *Lilium*. For convenience of reference the specific name used by the observer is retained.

ORDER.	SPECIES.	OBSERVER.
	Monocotyledones.	
Liliaceae	<i>Lilium Martagon.</i>	Nawaschin.
		Guignard.
	<i>L. pyrenaicum.</i>	Guignard.
	<i>Fritillaria tenella.</i>	Nawaschin.
	<i>F. Meleagris.</i>	Guignard.
	<i>Tulepa Gesneriana.</i>	Guignard.
	<i>T. Celsiana.</i>	Guignard.
	<i>T. sylvestris.</i>	Guignard.
	<i>Endymion nutans.</i>	Guignard.

ORDER.	SPECIES.	OBSERVER.
<b>Monocotyledones.</b>		
Orchidaceae	<i>Arundina speciosa.</i>	Nawaschin.
	<i>Phajus Blumei</i>	Nawaschin.
	<i>Phajus sp.</i>	Nawaschin.
	<i>Himantoglossum hircinum.</i>	Strasburger.
	<i>Orchis latifolia.</i>	Strasburger.
	<i>O. maculata.</i>	Strasburger.
	<i>O. mascula var. Hortii.</i>	Strasburger.
<b>Dicotyledones.</b>		
Ranunculaceae	<i>Caltha palustris.</i>	Thomas.
	<i>Delphinium elatum.</i>	Nawaschin.
Compositae	<i>Helianthus annuus.</i>	Nawaschin.
	<i>Rudbeckia speciosa.</i>	Nawaschin.
Monotropeae	<i>Monotropa Hypopitys.</i>	Strasburger.

The total number of species in which double fertilization has been recorded is thus twenty. These twenty species represent twelve genera (if *Himantoglossum* be included with *Orchis*), and five orders, three of which are Dicotyledonous.

Before we can properly estimate the collective value of the evidence, the difficulties of this branch of research must be considered. Material can be collected only in the flowering season. Excluding the four species observed before 1899, and the four collected and observed by Strasburger in 1900, we have twelve species belonging to nine genera, and representing four families—two of them Dicotyledonous—as the result of one season's pickling by three observers. The material for such work must be chosen from plants which are known to seed freely. The flowers must commonly be collected at least twice a day for two or three successive days, in order to secure the right stage, and each picking must be kept separate from the others throughout all the processes of the elaborate fixing methods, which are necessary in order to secure adequate preservation of the embryo-sac. When observation of material thus collected and preserved begins,

the preliminary work of cutting ovaries of different ages, and looking over the series of sections to determine which contain embryo-sacs at the exact point of development, is in itself heavy. Outside the Liliaceae and their allies we rarely find ovules arranged in rouleaux, and must sometimes sacrifice a whole ovary to secure median sections of a single ovule. Nor are adjacent ovules necessarily in the same phase of growth: fertilized and unfertilized are found in the same ovary, and among the fertilized some are too young and some too old for our purpose. In many cases it is essential to work with microtome sections only: in all they must be used to determine the minute structure with precision. But there are disadvantages in sections so thin as those of microtome series. The embryo-sac is commonly cut into many slices, of which each must be observed with a very high power, and the solid body mentally reconstructed from the succession of layers. The labour of thus observing a number of embryo-sacs, the sections of which are found in groups among long series of useless sections extending over many separate slides, and of indexing them in order to record such observations, can hardly be exaggerated.

Nor is the interpretation of results without difficulty. In some of these species the generative nuclei do not show the vermiform shape which makes it easy to distinguish them from the other nuclei in the embryo-sac. This is the case, for example, in *Endymion nutans* (Guignard, 4). Both the generative nuclei in this plant are small and egg-shaped. In some species, too, the polar nuclei are completely fused into one by the time the pollen-tube enters the embryo-sac. Now in plants which combine these features the identification of the second generative nucleus must of necessity be laborious. In the first place, a number of preparations from an earlier stage of development will be required to show that the fusion of the polar nuclei is commonly complete before the entrance of the pollen-tube. Even when this is established, a single preparation showing the fusion of two similar nuclei in the centre of an embryo-sac, which also contains a fertilized egg-



cell, is not by itself conclusive, for there is always the possibility that the polar fusion may have been retarded in any one instance. We require either a large number of such preparations to establish a strong probability that one of the fusing nuclei is generative, or several instances in which both generative nuclei are free in the embryo-sac, each approaching its proper goal. For before conjugation the generative nuclei can commonly be distinguished from those surrounding them by their smaller size and deeper stain. But such preparations are exceedingly rare: the passage of the generative nuclei from the pollen-tube to the nuclei with which they are to fuse must be tolerably rapid, for they are seldom caught on the way. Thus it may easily happen that thousands of sections must be passed in review, and hundreds carefully examined under a high power, before the point in question is finally determined.

Such difficulties may be prohibitive, and hence they greatly limit the choice of material. It is not surprising that in some cases only negative results have been reached (Merrel on *Silphium*, 31). Rather we may conclude that the number of widely-separated species in which double fertilization has been observed in so short a time, affords strong evidence that the process is general among Angiosperms which show the usual structure of the embryo-sac before fertilization. I think we are justified in assuming its existence in any such embryo-sac as a working hypothesis until we have the opportunity of settling the question by direct observation, and that the negative evidence must be very strong which should lead us to doubt it.

## II. MOVEMENT OF THE GENERATIVE NUCLEI.

The elongated and twisted shape of both generative nuclei in the embryo-sac of *Lilium Martagon* suggested to the original observers the arrest of automatic movements by the action of the fixing agent. This led to comparison with the newly discovered spermatozoids of *Cycas* (Ikeno, 25, 26),

*Ginkgo* (Hirase, 24), and *Zamia* (Webber, 41, 42, 43), and the generative nuclei were represented as much reduced spermatozoids, the existence of which formed a fresh phylogenetic link between the Cycadeae and Angiospermae. This hypothesis has been discredited by later work. The absence of cilia could be explained away so long as the vermiform nuclei had not been seen outside the embryo-sac, for Guignard pointed out (5, p. 375) that the cilia of antherozoids disappear as soon as they enter the archegonium. But in *Silphium* (Merrel, 31, p. 113, Fig. 61) the elongated male nuclei have been observed in the pollen-grain without any trace of cilia. Moreover, the small quantity of cytoplasm which enters the embryo-sac with each generative nucleus is in some cases at least quite undifferentiated (Guignard, 5, p. 375, Figs. 14, 16, 18), Guignard himself has shown that in *Endymion nutans* (4, p. 195, Figs. 21, 22, 23) the generative nuclei do not assume the vermiform shape. Thus in a plant so nearly allied to *Lilium* as this, the only character which suggested automatic motion is absent, and this fact in itself affords a strong reason for attaching no phylogenetic importance to the shape of the nuclei.

Finally, Strasburger has once again followed the whole process of fertilization in the living ovule of *Monotropa*. His results, so far as they affect our point, are briefly these. The polar nuclei are connected with the egg-apparatus and with the antipodal cells by thick strands of cytoplasm which are in constant streaming movement. The contents of the pollentube when discharged reach down to the egg-apparatus. One generative nucleus can be identified as soon as it touches the nucleus of the egg-cell. The other cannot be distinguished with certainty until it has been in contact with the central nucleus of the embryo-sac for some time. But it is almost certainly carried down by the current in the strand which joins the egg-apparatus to the central nucleus, and a swelling in this strand probably indicates its passage. Fixed and stained preparations confirm these observations. Both generative nuclei can be recognized in such preparations from the

time they leave the pollen-tube. In shape they are ovoid as in *Endymion* (Strasburger, 11, pp. 298–301).

On the other hand Nawaschin (8, p. 228, Fig. 1) has shown that the generative nuclei in the embryo-sac of *Helianthus*—and to a less marked degree those of *Rudbeckia*—are shaped like a corkscrew, and their appearance does strongly suggest that they possess the power of independent motion.

From the sum of these observations we may conclude that possibly the generative nuclei in some species of Angiosperms may make their own way through the embryo-sac to the nuclei at which they aim, but that in others this passage is almost certainly effected by the streaming motion of the cytoplasm in the embryo-sac, which carries the nuclei passively with it. If this be so, the power of automatic movement would seem to be an adaptive character, assumed or laid aside by the generative nuclei according to circumstances. These nuclei are indeed homologous with the nuclei contained in the spermatozoids of Vascular Cryptogams and Cycads, but they do not appear to be accompanied by any vestiges of the differentiated cytoplasmic structure characteristic of those bodies.

If we call the generative nuclei of *Helianthus*, for example, spermatozoids or antherozoids, we imply that the differentiated cytoplasmic element in such structures is not essential. This amounts to re-defining the terms, a serious step to take in order to emphasize a phylogenetic theory which is, to say the least, very doubtful. If we extend either term to include such non-motile nuclei as those of *Monotropa*, and probably those of *Endymion*, *Tulipa*, and others, we further give up the character of automatic motion, and it is difficult to see what is retained to distinguish a spermatozoid from a male cell or even from a male generative nucleus. This applies equally to the spermatia of Florideae (Guignard, 4, pp. 375–6 footnote).

### III. HOMOLOGY OF THE EMBRYO-SAC AND ITS CONTENTS.

One of the greatest gaps in our knowledge of vegetable morphology lies in our ignorance of the pedigree of Angio-



sperms. We have no series of forms to link the structures produced within the embryo-sac of such a plant as *Lilium Martagon* with those derived from the macrospore of the higher Cryptogams. The connexion of the Gymnosperms with the Vascular Cryptogams can be traced in their gametophytes. But though the ancient and very distinct groups included under the general term Gymnosperms are in some sense intermediate between Vascular Cryptogams and Angiosperms, yet our knowledge of embryo-sac structure in anomalous Angiosperms is so incomplete that we can hardly do more than hazard a guess as to whether any particular deviation from the best-known type is to be considered a specialized, a reduced, or a primitive form. Thus the attempt to derive Angiosperms from one or more primitive forms related to one or more groups of existing Cryptogams must be made at present without the aid of independent evidence as to the origin and progressive reduction of the Angiospermous gametophyte. But this is the very class of evidence which has been found most valuable in tracing the origin of any group of Gymnosperms, for example, from some 'generalized' form of Vascular Cryptogam. The gametophyte of some species of *Gnetum* is little less reduced than that of the typical Angiosperm. But the work of Karsten (28) and Lotsy (30) enables us to trace its reduction step by step through the successive forms of *Gnetum Gnemon*, *Welwitschia*, *Ephedra*, from a massive tissue such as that found in *Cycas* or the Coniferae. From that point the connexion with the prothallus of the higher Cryptogams is clear.

Until some causeway such as this has been built up stone by stone to connect the Angiosperms with their neighbours, all theories as to the race-history of the structures contained in the embryo-sac of *Lilium*—to take the classical example—must be considered as at best working hypotheses, useful indeed to stimulate and direct research, but without authority, and in their very nature tentative.

Without such hypotheses research could not proceed. Where two or more explanations of the same facts are equally

possible, the attention of the investigator is led to critical observations which may decide between them. His powers are directed to clearing up certain obscure points one after the other, and so step by step he wins his way to the truth. But any one of these explanations, if received too hastily as representing facts, will be fatal to research. Each may become the watchword of a school, and arouse fruitless controversy. It is therefore with no idea of coming to any decision in favour of one view rather than another that I propose to discuss the suggestions already made as to the homologies of the cells derived from the Angiospermous embryo-sac. I would rather ask what line of research is suggested by the work of the last decade in this direction, and in particular what change in theoretical conceptions has arisen from the discovery of double fertilization.

We have recently been learning by degrees that the structure of the embryo-sac in Angiosperms is by no means so uniform either before or after fertilization as botanists were once apt to believe. Some of these variations are undoubtedly derived from the more usual structure, and are specialized for some particular end, rather than primitive. Thus the persistence and occasional division of the antipodal cells in *Nigella sativa* described by Westermaier (44, p. 8), is probably due, as he suggested, to their function in this plant as transmitters of food material. But it would be very rash in the absence of further evidence to assume that the multiplication of antipodal nuclei in the embryo-sac of *Sparganium simplex* (Campbell, 19, 20), must be referred to a similar cause. Westermaier himself has described a growth of 'antipodal tissue' in *Zea Mays* and other Grasses before fertilization. In *Sparganium*, and perhaps in *Zea* also, the multiplication of cells within the embryo-sac cannot but suggest the formation of a prothallus. Whether this suggestion is justified can be determined only by extended research.

The antipodal cells have been considered as representing (*a*) the vegetative part of the prothallus, (*b*) a second egg-apparatus, and (*c*) that part of a monoecious prothallus which

produces the male elements. This last view is implied in Le Monnier's explanation of the fusion of the polar nuclei as a sexual act (see Groom, 22, p. 133). The upper polar nucleus must, as the sister of the egg-nucleus, represent the female element in the conjugation. The lower polar nucleus must therefore represent the male element. But this assumption is no longer necessary, as we now know that there is a triple fusion, in which the second generative nucleus supplies the male element. Thus the third suggestion (*c*) has been shelved, and we are left with two.

There is nothing in the discovery of double fertilization to decide between the two views which remain. Future research may reveal a chain of forms linking the whole structure of the embryo-sac before fertilization with some more primitive type in which the homologies with lower plants are clear. Many species are known in which the antipodals increase in number. For references to the literature of the subject I must refer to Strasburger's paper (11, p. 311). As suggested above, there is no reason to suppose that all these forms are adaptive because some are so.

The discovery of double fertilization has, however, a direct bearing on the vexed question of the origin of the Angiospermous endosperm. This tissue was considered by Hofmeister to represent a prothallus, the development of which was arrested before fertilization. The fusion of the two polar nuclei which takes place about the same time as the fertilization of the egg-cell gave the signal for renewed development. This view held its ground for a generation, and gave rise to the accepted nomenclature. The food-tissue formed by Gymnosperms within the embryo-sac before fertilization, which undoubtedly represents the female prothallus, is generally called the endosperm to mark its supposed homology with the tissue we are now considering in Angiosperms. Strasburger (11)—himself a supporter of the classical view—has justly remarked (p. 314) that the true issues would be rendered clearer if we called the Gymnospermous tissue a prothallus, and confined the term endosperm to Angiosperms. This is a



very welcome suggestion, and I hope it will find favour with botanists.

Le Monnier (29), on the other hand, suggested in 1887 that the fusion of the polar nuclei ought to be considered as an act of fertilization, and the tissue derived from it as a second embryo modified to serve as a food-tissue for the embryo regularly derived from the fertilized egg-cell.

The fact that a generative nucleus takes part in the formation of the original endosperm-nucleus certainly strengthens the view that this nucleus is formed by an act of fertilization. Whether the tissue derived from its repeated division is morphologically a second embryo or not is a question of race-history. It must be settled by purely morphological evidence—by the search for intermediate forms, which may either give some conception of the stages through which such a twin-formation passed before it assumed its present aspect, or may link the endosperm with a reduced prothallus. Treub (40, p. 194) has given good reason for supposing that a considerable number of nuclei are formed in the embryo-sac of *Casuarina* before fertilization. It is of the first importance that we should know the details of the process of fertilization in this genus. Unfortunately the difficulties of observation are great (Treub, 40, p. 199). They may possibly be overcome by the application of the most recent fixing and staining methods.

In *Peperomia*, Johnson (27) has found a fusion of six nuclei to form the primary endosperm nucleus. His work and that of Campbell (21) is discussed by Strasburger, who is inclined to consider the structure of this embryo-sac as derived from the normal structure rather than as primitive. The examination of more allied forms might throw light on the question.

The embryo-sac of the Amentiferae shows the usual arrangement of nuclei before fertilization. (Nawaschin, 33, 34, 35, 36, and Benson, 17). Much in the development of the embryo-sac, however, shows a primitive structure, and it would be very desirable to have fuller details of the act of fertilization itself.

Strasburger (11, p. 310) has suggested that the Angiosperms

postpone the formation of an endosperm until fertilization is accomplished in order to avoid the waste of material which must occur if this massive tissue were thrown off by the plant with every unfertilized ovule. This is a very probable explanation, but it does not affect the morphological question at all. For the plant in need of a food-tissue, to be formed after fertilization, is as capable of adapting a modified second embryo to that purpose as of postponing the formation of a prothallus. In the latter case indeed the expedient of a second conjugation to stimulate development appears cumbersome. In some species of *Gnetum* (*Gn. Rumphianum* and *Gn. ovalifolium*, Karsten 28), the formation of a prothallus begins before fertilization, is checked for a time, and proceeds after fertilization, without any fusion between a generative and a prothallial nucleus. Both generative nuclei in fact conjugate with egg-nuclei, and form two rudimentary embryos. Here the stimulus which excites the prothallus to renewed activity must be carried in some other way.

In short, the problem from the morphological point of view is this. We have to account historically for the endosperm of Angiosperms. If it arose from a belated formation of prothallus, we must trace the origin of the triple nuclear fusion which precedes its development. Such a fusion is an interpolation in the history of the prothallus which needs explanation. If, on the other hand, the endosperm is a modified embryo, derived from a fertilized egg-cell, we have to account for the interference of the lower polar nucleus with the act of fertilization, and for the subsequent development of a body unlike a normal embryo.

As regards the last point it is perhaps worth notice that the common mode of endosperm-formation among Angiosperms recalls that of the pro-embryo in *Cycas circinalis* (Treub, 39, p. 5, figs. 9, 12, 13, Pl. I) and *Stangeria paradoxa* (Lang, 28 a, p. 293). That the pro-embryo is formed within an archegonium, and the endosperm within the embryo-sac, is of less importance than appears at first, for the archegonium is completely suppressed among Angiosperms.

## IV. NATURE OF THE TRIPLE NUCLEAR FUSION.

The fourth and last question to be dealt with is whether the fusion of the second generative nucleus with the two polar nuclei can be considered as a true act of fertilization.

The expression fertilization may be used in an abstract or a concrete sense. In the abstract it denotes the process by which characters from two individuals are transmitted to a single organism in the succeeding generation. This phenomenon is almost universal throughout the animal and vegetable kingdoms, and its effects have been observed by many successive generations of breeders both of animals and of plants. In this way a considerable body of evidence has accumulated, and it has been found that certain laws are universally true of organisms which thus spring from a double stock. Such an organism passes through its complete life-history, which may include more than one cycle of development. It exhibits a combination of characters drawn from both parents. The offspring of the same pair differ from each other: some resemble one parent, some the other, and those of mixed appearance may lean to either side. But a balance is maintained in each generation between the two stocks, so that neither parent has on the whole greater weight than the other.

Two features may be commonly distinguished in the abstract conception of the process of fertilization: the union of the characters of both parents, at the same time that a certain balance is maintained between them, and the impulse to further development impressed on members of the younger generation (Strasburger, 11, pp. 304-6; R. Hertwig, 23 *a*, p. 149). The combination and balance of parental qualities has been happily called 'amphimixis' by Weismann (45, pp. 20 and 232).

In many unicellular organisms, no special stimulus to development seems to accompany the act of conjugation (Weismann, 45, p. 232; R. Hertwig, 23 *a*, p. 146). But among the higher plants, with which alone we are concerned at



present, a double process is included under the general term fertilization. Characters from both parents are transferred to the offspring by amphimixis, and the development of the fertilized egg-cell is secured by the presence of a growth-stimulus.

If we now inquire into the machinery by which these results are attained, we find that the act of fertilization is remarkably uniform in all organized beings. It consists in a fusion of two cells to form a new one, which then divides and ultimately gives rise to an individual whose characters are intermediate between those of its parents. This is fertilization in the concrete sense. Its phenomena supply a material basis for the general laws of heredity to which I have just referred.

When the act of fertilization is studied in detail, certain features are found to be general. The male cell is usually small, and the nucleus forms the most considerable part of it. The cytoplasmic elements are greatly reduced. When the male and female cells fuse, the union of their nuclei is the most characteristic feature of the process. Both are generally in the resting state at the time of fusion, but though they commonly unite in that condition so far as to enclose a single cavity, yet it very often happens that fusion is not complete until the first karyokinesis has taken place<sup>1</sup>. The male and female chromosomes can frequently be distinguished in the equatorial plate, and it is not until a segment of each has travelled to either pole, and the daughter-nuclei are in process of construction, that the chromatic elements from the male and female nuclei are really united. Boveri (18) does not consider the act of fertilization complete until the first karyokinesis after conjugation is accomplished (p. 416). There is a double advantage in adopting this view. We are secure that the chromatin-fusion has actually taken place, and the occurrence of the first division in the egg shows that the stimulus to development is also present.

The arguments which have led most biologists to consider

<sup>1</sup> See the discussion in Boveri's article on Fertilization (18, pp. 403-5), and the literature there referred to. Also Guignard (23, p. 199).

the union of chromosomes as the agency by which the parental qualities are united in the offspring, are too familiar to need repetition. I need only refer to Boveri's essay on Fertilization (18, pp. 417-23) and Weismann's *Germ-Plasm* (45, pp. 23-35). We must consider the process of amphimixis as bound up with the act of nuclear conjugation.

It is less easy to localize the agent which carries with it the impulse to growth. In some animals it appears to reside in the centrosome belonging to the sperm-nucleus (Boveri, 13, pp. 427-9)<sup>1</sup>. Strasburger (11, p. 307) conjectures with much probability that in the higher plants the male cytoplasm may carry the growth-stimulus with it. If this be so, we have in the higher plants a compound process which it seems most convenient to call fertilization, including two subordinate processes. These are (1) amphimixis, or the union of parental qualities in the embryo, and (2) a growth-stimulus which ensures that development of this embryo shall at least begin. Fertilization thus defined is effected by the fertilizing act, which also includes two distinct phenomena—nuclear conjugation and union of the cytoplasmic constituents. There is good reason to suppose that the process of amphimixis is inseparably connected with the act of nuclear conjugation, and very possibly there may be a similar connexion between the growth-stimulus and the fusion of male cytoplasm with that of the egg-cell.

We have hitherto been considering the fertilization of the egg-cell by the male cell from the pollen-tube, the result of which is a normal embryo. Let us now apply the same touchstones to the triple fusion—often regarded as a second act of fertilization, which produces endosperm-tissue. Here we have an undoubted male nucleus fusing with two others. Of these the upper polar nucleus is the sister of the egg-nucleus. It can hardly be regarded otherwise than as a female nucleus. There is in this union then, a male and a female element. Both are perfectly normal, formed by a series of reduction-divisions, and containing the reduced

<sup>1</sup> See also an article in 'Nature' for April 5, 1900, p. 551.

number of chromosomes. So far we have a typical sexual union. But the third element in the fusion upsets the balance. The lower polar nucleus, though originally derived from the first division of the embryo-sac nucleus, which is a reduction-division, has since acquired a larger number of chromosomes. In *Lilium Martagon*, for example, the two male generative nuclei—the nucleus of the egg-cell, and the upper polar nucleus—are each built up of 12 chromosomes. The lower polar nucleus, however, is built up of at least 24 chromosomes, sometimes of more. (Guignard, 23, pp. 187-8; Sargent, 37, pp. 465 and 468.)

The nuclear fusion then which produces the first endosperm-nucleus differs from the normal conjugation of the sexual nuclei by the presence of a large mass of chromosome-substance derived from a nucleus which shows no sexual differentiation. The fusion is certainly accompanied by a growth-stimulus, for the compound nucleus divides very rapidly.

In the sea-urchin (Boveri, 18, pp. 425-6) more than one sperm-nucleus can be forced to unite with a single egg-nucleus. Compound spindles are then formed, and the larval structures derived from the division of such ova are monstrous. It seems, however, that a normal larva is sometimes produced as a consequence of the union of two sperm-nuclei with one egg-nucleus. In such a case the number of chromosomes in the nucleus of the fertilized ovum would be one-half in excess of the usual somatic number, or three times the reduced number. But the first endosperm-nucleus of *Lilium Martagon* contains at least twice the somatic number of chromosomes, or four times the reduced number. Many observers have noticed the large number of chromosomes characteristic of dividing nuclei in endosperm-cells.

The primary endosperm-nucleus formed by the triple nuclear fusion just discussed, gives rise on division, not to a normal embryo, which will pass through all the stages in the life-history of its species, and ultimately grow into a complete plant, but to an endosperm, which is a comparatively



small and short-lived mass of tissue. Sooner or later the endosperm is consumed by the growing embryo. Thus the result of the triple fusion differs from that of a normal act of fertilization. The organism derived from it is not a complete plant. Reproductive cells formed by a true fertilizing act are distinguished, as we have seen, by two features. They give rise to an organism which (1) passes through the complete life-history of the species to which it belongs, and (2) combines in itself some characters from both parents.

The endosperm will not stand the first test, but it has been found to satisfy the second. H. de Vries (14) and Correns (1, 2) have shown that when one variety of the Maize is crossed with another which differs from it in the colour or chemical constitution of its endosperm, the endosperm of the offspring commonly shows hybrid characters. Both observers believe that this is due to the presence of a male nucleus in the nuclear fusion which forms the primary endosperm-nucleus.

This view has not escaped criticism. Nawaschin (8, p. 230) supports it in a thorough-going way. He considers that these observations have conclusively shown the triple nuclear fusion to be a true act of fertilization. Correns (1), followed by Marshall Ward (15) in a summary and criticism of the subject in 'Nature,' observes that another view is possible. The embryo itself might exercise an influence on the endosperm, as, for example, by the formation of an enzyme, Strasburger (11, p. 308), though opposed to the extreme view that the triple fusion is in all essentials an act of fertilization, throws no doubt on the suggestion that the paternal characters in the hybrid endosperm are due to the presence of a male element in the nucleus from which that endosperm is derived. H. J. Webber (16) has lately published extended researches on the subject<sup>1</sup>. He finds that the endosperm does not always show hybrid characters, even though the embryo in the same embryo-sac may do so, and suggests that in such cases the second generative nucleus may not have reached the

<sup>1</sup> Dr. Webber's paper is referred to in 'Nature,' vol. 62, p. 601. I quote from the abstract given there, as I have not seen the original.

endosperm-nucleus at all. In other cases where the endosperm resembles the male parent in certain parts only, he supposes the second generative nucleus to have divided independently.

It would certainly be desirable to have independent evidence that the triple fusion normally takes place in *Zea Mays*. Until such has been obtained I think we may assume that it does so. It has been shown earlier in this paper that the mass of evidence for its general occurrence among Angiosperms is considerable. Dr. Webber's views, though quite tenable, hardly seem justified by his observations (as quoted in 'Nature'), for we know how capricious the facts of heredity appear in particular cases. The balance of probability, as of authority, seems to me to be in favour of regarding the paternal endosperm characters as derived from the presence of a male element in the primary endosperm-nucleus.

Returning to the question in hand, we have found that the nuclear fusion under consideration resembles a sexual union in containing a normal male and female element, and differs from it in including a third nucleus which in some cases is built up of at least twice as many chromosomes as either. The result of this fusion is a body which often resembles the product of fertilization in exhibiting a mixture of parental characters, but is unlike such a product in form and life-history. Whether we shall call such a fusion a true act of fertilization or not depends chiefly on our theoretical conception of the *process* of fertilization, and the definition of the *act* of fertilization which we choose to adopt. Authorities have already differed on this point. Before discussing the grounds on which they base their respective views, I wish to make a suggestion as to the meaning to be attached to the presence of a third nucleus.

The observations already quoted on polyspermy in the sea-urchin have shown that the union of more than two nuclei in the sexual act commonly produces monstrous forms. Groom (22) has shown that fusions of two or more asexual nuclei among Fungi often led to the production of degenerate

tissue. In the case we are considering, we have a triple fusion which unites in a single nucleus a mass of chromosome-substance at least twice as great as that found in an ordinary somatic nucleus. The body formed by repeated division of this nucleus is small and short-lived, though from the presence of male and female elements in the combination we might almost have expected the formation of a second embryo.

It is difficult to avoid the suspicion that the presence of the third nucleus with its redundant chromosomes serves to secure the degeneracy of the resulting tissue. This hypothesis does not depend on any view as to the race-history of the fusion. If it is historically a fertilization the third nucleus may have been introduced to maim the second embryo from the beginning, and secure the survival of the first without a struggle. Its value as a food-body would secure the persistence of the abortive embryo in the shape of the tissue we call endosperm. If, on the other hand, the second generative nucleus served from the outset merely as a signal to one of the polar nuclei that fertilization had been accomplished, and that it was time for prothallial formation to proceed, the intrusion of a third nucleus would be necessary to prevent the formation of a second embryo as a consequence of the signal.

Although the question now under discussion is purely physiological, its solution would undoubtedly be forwarded by any advance in our knowledge concerning the origin of the triple fusion. This is another very strong reason for the investigation of anomalous embryo-sacs in the hope of hitting on primitive forms. If the first endosperm-nucleus is formed by a nuclear fusion which has once been a normal sexual union, it must now be considered as a more or less degenerate act of fertilization, and the question whether it has retained enough of its original nature to still deserve the name of fertilization is a comparatively simple one. But if such morphological research should show that Strasburger's view is justified, that the generative nucleus has never been a fertilizing agent, but has served merely as a signal for the further development of the prothallus, the process will appear in a different light. The



resemblance to a true act of fertilization must then be considered as accidental.

Strasburger (11, p. 308) has expressed this view with great force. It appears to him inevitable that the presence of the male element in the original nuclear fusion should affect the character of the tissue derived from it, as shown by de Vries and Correns, but he does not regard this as a proof of true 'generative fertilization,' or in other words of amphimixis. On the contrary, he proposes to classify the triple fusion among the phenomena of 'vegetative fertilization,' or, as I have hitherto called it, growth-stimulus.

It is clear that this proposal is based on a double assumption. In the first place, Strasburger considers that the endosperm is phylogenetically a prothallus, and that the nuclear fusion which gives rise to it has never been a true act of fertilization, or to use his own term a 'generative fertilization.' In the second place he believes that there is no purpose in the transference of paternal characters to the endosperm. Even if it should be proved later that such transference originally began at a time when the endosperm was clearly a second embryo, and the conjugation giving rise to it a normal act of fertilization, he believes that the process has now degenerated so far that it is physiologically a mere stimulus to growth (l. c., p. 308). On this view the presence of paternal characters in the endosperm is either an accident inseparable from the mode of stimulus which provokes its formation, or a mere survival with no physiological meaning at the present time.

We must wait for the solution of the historical question. But no one, so far as I am aware, has as yet suggested that the transference of paternal characters to the endosperm may still be of advantage to the plant. This suggestion—which seems to me of great importance—I owe to Miss Thomas, who has herself demonstrated the existence of 'double fertilization' in *Caltha palustris*, (Thomas, 12, 13). I am indebted to her for permission to publish her hypothesis here for the first time.

Miss Thomas' suggestion is that in cases of cross-fertilization the endosperm formed as the result of a nuclear fusion which includes a male element—the twin-structure of the male element in the fertilized ovum—may be more suitable for the nourishment and development of the embryo arising from the same mixed stock than an endosperm formed from the mother plant alone. The food-material, for example, contained in the cells of the crossed endosperm might agree better with the crossed embryo than that supplied by an endosperm of purely maternal origin. This appears the more probable as we know that one of the characters in which de Vries and Correns traced the influence of the pollinating Maize-plant was the presence of sugar within the endosperm-cells in place of starch.

But if the endosperm is more fitted for its work by the presence of mixed characters, we must consider the phenomena of amphimixis as essential to the process of nuclear fusion which gives rise to the endosperm. In this case we can hardly refuse to regard it as now a true act of fertilization, however it may have arisen.

It appears to me possible that this view of the increased utility of the endosperm of mixed origin may partly account for the surprising effect of cross-fertilization in the first generation of plants remarked on by Darwin. He speaks of the good effects of cross-breeding as appearing much sooner in a race of plants than in one of animals. If the food supplied to the embryo is rendered more nourishing at the same time that the embryo itself gains in vigour, the effect of a cross would be doubled in a single generation.

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## NOTES.

ON THE OCCURRENCE OF A SEED-LIKE FRUCTIFICATION IN CERTAIN PALAEOZOIC LYCOPODS<sup>1</sup>. By D. H. SCOTT, M.A., Ph.D., F.R.S.—It has generally been assumed by palaeobotanists that the fossil seeds described by Williamson<sup>2</sup> under the name of *Cardiocarpon*, even if not necessarily co-generic with the *Cardiocarpus*<sup>3</sup> of Brongniart, at least belonged to the same group of Gymnospermous plants<sup>4</sup>. Brongniart's specimens, often preserved with marvellous perfection, have proved to be the seeds of members of the extinct Order Cordaiteae, or of allied plants. The same conclusion applies to certain of the British forms, notably the *Cardiocarpon anomalum* of Carruthers<sup>5</sup>, which was certainly Cordaitean, and probably to some of Williamson's examples.

The specimens to be shortly described in the present note show, however, that seed-like bodies, identical with those figured by Williamson under the name of *Cardiocarpon anomalum*<sup>6</sup>, were borne on Lepidodendroid cones, otherwise indistinguishable from *Lepidostrobus*. They thus prove that under the genus *Cardiocarpon*, and even under the 'species' *C. anomalum*, totally different objects have been confounded, namely, the seeds of Cordaiteae or Cycads on the one hand,

<sup>1</sup> From the Proceedings of the Royal Society, Vol. lxvii.

<sup>2</sup> Organization of the Fossil Plants of the Coal-measures, Part VIII, Phil. Trans., Vol. clxvii, Part I, 1877, p. 254.

<sup>3</sup> Founded in Brongniart's Prodrôme d'une Histoire des Végétaux Fossiles, 1828. The forms *Cardiocarpon* and *Cardiocarpus* have been used indiscriminately by authors.

<sup>4</sup> See, for example, Solms-Laubach, Introduction to Fossil Botany. English edition, p. 120.

<sup>5</sup> Notes on some Fossil Plants, Geol. Mag., Vol. ix, 1872.

<sup>6</sup> loc. cit., Part VIII, Plate 14, Fig. 118, and Plate 16, Fig. 119; Part X, 1880, Plate 20, Fig. 64. These figures are from specimens which I have certainly identified with the Lepidostroboïd fructification. Others figured by Williamson are of doubtful nature.

and the integumented megasporangia of certain Palaeozoic Lycopods on the other. The latter organs present close analogies with true seeds, but are wholly distinct in detailed structure from the Gymnospermous seeds above mentioned.

The discovery of the specimens of the new cone is due to Messrs. J. Lomax and G. Wild, who recognized it as a *Cardiocarpon*-bearing strobilus, resembling a *Lepidostrobus*<sup>1</sup>.

The original specimens, which are calcified and generally well preserved, were derived from the Ganister beds of the Lower Coal-measures, some from Hough Hill, Stalybridge, others from Moorside, Oldham. Numerous sections were cut by Mr. Lomax and Mr. Wild. A closely similar fructification occurs, at a much lower horizon, in the Burntisland beds of the Calciferous Sandstone Series.

The strobilus is of the ordinary *Lepidostrobus* type. The cylindrical axis bears numerous spirally disposed sporophylls, each of which consists of a long horizontal pedicel, expanding at the distal end into a rather thick lamina, which turns vertically upwards.

Anatomically, the structure is also that of a *Lepidostrobus*. The stele which traverses the axis has a narrow ring of centripetal wood, and a large pith; the leaf-trace bundles which pass out to the sporophylls are collateral in structure, and agree closely with those described by Mr. Maslen in *Lepidostrobus Oldhamius*<sup>2</sup>.

The ligule is sometimes well preserved; it is seated in a depression of the upper surface of the sporophyll, at the distal end of the porangium, and is thus in the normal position<sup>3</sup>.

With one exception, the specimens of the strobilus are immature, and their tissues not quite fully differentiated. These younger specimens bear sporangia which are essentially those of a *Lepidostrobus*. A single large sporangium is seated on the upper surface of the horizontal pedicel of each sporophyll, to the median line of which it is attached along almost its whole length.

The sporangium narrows out towards the top, and terminates above in a well-marked ridge; in general form it resembles Williamson's *Cardiocarpon anomalum*, but in the immature condition there is no

<sup>1</sup> See the note by Messrs. Wild and Lomax, On a new *Cardiocarpon*-bearing Strobilus, Annals of Botany, March, 1900.

<sup>2</sup> Maslen, The Structure of *Lepidostrobus*, Trans. Linn. Soc., London, Ser. 2, Vol. v, 1899.

<sup>3</sup> Maslen, The Ligule in *Lepidostrobus*, Annals of Botany, Vol. xii, 1898.



integument. The outer layer of the sporangial wall has the columnar or palisade-like structure characteristic of *Lepidostrobis*; it is lined by a more delicate inner layer, which may be several cells thick.

So far the structure is simply that of a *Lepidostrobis* with rather thick-walled sporangia.

Within the sporangial cavity, the membranes of the megaspores are usually preserved; a single large megaspore almost fills the sporangium, but smaller, abortive spores, with thicker walls, are also present. Some specimens show that three of these abortive spores were present in each sporangium. It appears, then, that a single tetrad was developed in each megasporangium, and that of the four sister-cells one only came to perfection, constituting the functional megaspore.

In one specimen, discovered by Mr. Wild, the strobilus is in a more advanced condition. In its upper part the sporophylls simply bear sporangia, as above described, but lower down in the cone these are replaced by integumented, seed-like structures, identical with the detached bodies called *Cardiocarpon anomalum* by Williamson.

The structure of this strobilus is sufficiently well preserved to show that the anatomy of the axis agrees with that of the less mature specimens, and, as the tissues are more completely formed, exhibits the Lepidostroboid characters even more clearly.

Mr. Wild's specimen, then, demonstrates that the *Cardiocarpon anomalum* of Williamson was borne on a cone with all the characters of a *Lepidostrobis*, and that it represents the matured condition of the megasporangium and sporophyll.

The detailed comparison of specimens in the young and the mature condition has shown the nature of the change which converts the megasporangium, together with its sporophyll, into a seed-like organ. The nucellus of the latter retains almost unaltered the structure of the megasporangial wall, with its columnar layer. In the sporangial cavity the single large megaspore, accompanied by its abortive sister-cells, is present as before. A thick integument has, however, grown up from the sporophyll, completely overarching the megasporangium, except for a narrow crevice left open at the top. When seen in a section tangential to the strobilus as a whole, this crevice is cut across, and presents exactly the appearance of a micropyle; in reality it differs from a micropyle in being a narrow slit, extending almost the whole length of the sporangium, in the radial direction, whereas the micropyle of an ordinary seed is a more or less tubular passage.

The integument springs from the upper surface of the sporophyll-pedicle; it does not consist of the incurved margin of the pedicle, for, in the more distal region, the margin of the latter projects considerably beyond the insertion of the integument.

From the frequency of detached specimens in the *Cardiocarpon* condition, it appears that in nature the sporophyll, bearing the integumented megasporangium, was shed as a whole, though parts of the sporophyll-lamina no doubt perished, only so much being persistent as was necessary to form a complete envelope to the 'seed.'

In a strobilus associated with the seed-like specimens, and bearing microsporangia, it was found that the latter, like the megasporangia of the female cone, are provided with integuments. This specimen was figured by Mr. Maslen as a variety of *Lepidostrobus Oldhamius*<sup>1</sup>, though possibly deserving specific rank, a determination with which I agreed at the time. There is every reason, however, to suppose that this strobilus was a male fructification of the same species, the female of which bears the integumented seed-like megasporangia above described. The microsporangial integument is more widely open than that of the megasporangium.

The Burntisland specimens, which from their horizon are presumably of a distinct species, are at present only known in the isolated, *Cardiocarpon* condition. They are of interest for two reasons: in one specimen the ligule is clearly shown, enclosed by the integument, the only example of this organ, so far observed, in the mature, seed-like stage of the fructification.

Another of the Burntisland specimens is the only one as yet observed in which the prothallus is present<sup>2</sup>. It fills a great part of the functional megaspore, which is almost co-extensive with the sporangial cavity, and consists of a large-celled tissue, resembling the prothallus of *Isoetes* or *Selaginella*. The peripheral prothallial cells are smaller than the rest, but no archegonia could be detected.

The bodies described in this note resemble true seeds in the possession of a testa or integument, and in the fact that one megaspore or embryo-sac alone came to perfection; the seed-like organ was likewise shed entire, and appears to have been indehiscent. In many

<sup>1</sup> Maslen, Structure of *Lepidostrobus*, p. 371, Plate 37, Fig. 21.

<sup>2</sup> I have since examined a section, cut by Mr. Lomax from one of the Coal-measure specimens, in which the prothallus is even better preserved.—Note, added October 9, 1900.

points of detail, however, the reproductive bodies in question differ from the seeds of any known Gymnosperms; they afford no proof of the origin of the latter Class from the Lycopods. The newly discovered fructification nevertheless shows that certain Palaeozoic Lycopods, with strobili at first indistinguishable from *Lepidostrobus*, crossed the boundary line which we are accustomed to draw between Sporophyta and Spermatophyta.

As these fossils appear worthy of generic rank, I propose to found the genus *Lepidocarpon* for their reception; it may be briefly characterized as follows:—

*Lepidocarpon*, *gen. nov.*—Strobilus, with the characters of *Lepidostrobus*, but microsporangia and megasporangia each surrounded by an integument, growing up from the upper surface of the sporophyll. Megasporangium completely enclosed in the integument, except for a slit-like micropyle along the top. A single functional megaspore developed in each megasporangium. Sporophyll, together with the integumented megasporangium, detached entire from the strobilus, the whole forming a closed, seed-like, reproductive body.

It is proposed to name the Coal-measure form *Lepidocarpon Lomaxi*, and that from Burntisland *L. Wildianum*. Both were included by Williamson under his *Cardiocarpon anomalum*, which, however, is quite different from the seed so named by Carruthers.

A full illustrated account of these fossils is in preparation, and will shortly be submitted to the Royal Society.

**THE AFFINITIES OF THE MESOZOIC FOSSIL, BENNETTITES GIBSONIANUS, CARR.**—Carruthers<sup>1</sup>, in 1870, described and figured the general features of the vegetative axis of certain specimens of *Bennettites*. This was shown (as in *B. Saxbyanus*)<sup>2</sup> to be eminently Cycadean in character, and possessed a single vascular cylinder. But this fossil stem clearly exhibits a more primitive type of structure than that of the stem of modern Cycads, the two chief indications of this being (1) the course of the leaf-traces from the leaves to the central cylinder which, as in the most primitive parts of the axis of modern Cycads such as the primary node and the cone, is

<sup>1</sup> Carruthers, W., On Fossil Cycadean stems from the secondary rocks of Britain, Trans. Linn. Soc., Vol. xxvi, 1870.

<sup>2</sup> loc. cit., Plate 57, Fig. 3.



direct and radial, instead of girdle-like and tangential in direction; and (2) the large size and arc-shaped character of the leaf-traces as they leave the central cylinder, and the splitting up of these large leaf-bundles into numerous smaller ones as they pass into the leaf; this is essentially the phenomenon met with in the Medulloseae. The Bennettiteae, therefore, in the character of their vegetative stem, are more primitive than modern Cycads.

Carruthers, in the same paper, describes and figures the fructification of *Bennettites Gibsonianus*, Carr. The structure is briefly this: the short axes, arising in the axils of the vegetative leaves of the stem, terminate in what the author terms a 'cellular cushion'; this latter bears all over its upper surface great numbers of 'vascular cords,' each such 'cord' terminating at its apex in a seed. These 'cords' are completely enveloped, except for tiny orifices communicating with the apices of the seeds, by a 'pericarp' or a 'mass of irregular cellular tissue.' He regards the seeds as being borne on axial organs, as distinct from the foliar organs which bear the seeds of modern Cycads. The fruit is a compound one. The general conclusion is that 'in comparing the structure of this plant with the inflorescence of the recent Cycadeae, the points of difference are more obvious than those in which they agree.'

Further researches undertaken by Solms-Laubach<sup>1</sup> resulted in showing that the 'pericarp' of Carruthers consists of a number of appendages of the axis, probably of foliar nature, arising between the seminal 'cords' or on these 'cords' themselves which, protruding above the latter, overlap and form a dense protective covering for them. The whole fructification is itself enclosed within an involucre of long, lanceolate bracts arising below the 'cushion'.<sup>2</sup> Solms puts forward three views as to the morphology of the appendages: they may be either (1) all foliar organs, some being fertile carpels bearing a terminal ovule, the remainder sterile carpels; or (2) all the organs may be axial members; or, finally, (3) the seed-stalks may be axes, and the interstitial organs leaves. Carruthers did not, apparently, regard the seminal 'cords' as possessing a foliar nature, but considered the whole as an axial structure. Solms, while admitting the possibility of the seed-stalks being axial organs, is quite prepared to entertain the view that the entire shoot is 'a flower with numerous leaves, some of which

<sup>1</sup> Solms-Laubach, Bot. Zeit., 1890.

<sup>2</sup> Solms-Laubach, Einleitung in die Palaeophytologie, 1887, Fig. 5 A, p. 98.

are sterile and serve to form the involucre, while the others bear the ovules.'

In carefully pondering over the morphological character of the fructification of the fossil plant under consideration, I cannot resist the final conclusion that the view which regards the seminal stalks or 'cords' as being *foliar* in nature is the only admissible and correct one. Further, I adopt the view put forward by Čelakovský<sup>1</sup>, viz. that the sporophyll is originally and primitively *radial* in structural symmetry, bearing the sporangium in a *terminal* position.

This view is founded on the theory that all vascular axes with their appendages, the foliar organs, have had their origin in the sporogonium of a Bryophyte such as *Anthoceros*. This sporogonium is a structure of essentially *radial* symmetry; its earliest branches or appendages must necessarily also have possessed the same character. As sterilization proceeded apace in these radially-constructed appendages, the sporogenous tissue would become for obvious reasons projected towards the apex of the appendage and would thus in time come to represent a sporangium situated *terminally* to its radially-constructed sporophyll. This type of compound sporogonium or of strobilus is preserved for us in the female cone of *Ginkgo*, and in *Osmunda* and a few other Ferns. In modern Cycads there is a clear case of transition from the radial type of sporophyll to the later dorsiventral type, and in Angiosperms, at least on the female side, this transition has become complete, where the carpels are always dorsiventral in construction and bear the ovules marginally or ventrally. This latter type of sporophyll, therefore, is that which is found in the group of plants which stand highest on the evolutionary scale, and represents thus the type towards which all primitive radially-constructed sporophylls must evolve, for it is obviously the one best adapted for the purposes of fertilization and dissemination of the seed.

It is on account of the above views that I regard the fructification of *Bennettites* as being essentially *more primitive* in character than that of modern Cycads, in contradistinction to the view hitherto propounded that in the cone of *Bennettites* we see an advance, beyond that of modern Cycads, in the Angiospermous direction. In my opinion, no *advance* may rightly be spoken of except in connexion with those *essential* organs of a plant which have always formed the basis for the

<sup>1</sup> Čelakovský, Nachtrag zu meiner Schrift über die Gymnospermen; Engler's Jahrbuch, 1897.

classification, *on evolutionary lines*, of the great groups of the vegetable kingdom. Now the distinguishing feature of the great group, the Angiosperms, which to-day head the evolutionary line, is the character of one set of its essential organs, the female sporophylls or carpels, which are dorsiventral in structure and bear the ovules *marginally* or on their ventral surface, and are, moreover, closed together over the ovules to form the characteristic ovary. But *Bennettites* shows absolutely no indication of such an advanced structure in its essential organs, the sporophylls, which remain primitively *radial* in structure, bearing the ovules in a *terminal* position; the angiospermous condition (using this term as in no sense referring to the 'Angiosperms') being brought about solely by the *unessential* organs: the interstitial bracts or sterile sporophylls, and the large ensheathing bracts springing from the base of the entire cone; these together form an adequate protection for the seeds. That modern Cycadean plants still retain the primitive radial symmetry of their sporophylls here and there may be seen in the female sporophylls of Cycads generally (although in these the ovules have become relegated to a marginal, or rather a sub-dorsal position), in the radially-constructed short ovuliferous segments of the female sporophyll of *Cycas*, which bear the ovules in a terminal position, and in the female cone of *Ginkgo*, which, as clearly observed in abnormal cases, produces the ovules terminally on radially-constructed sporophylls.

The conclusion which is, therefore, to be drawn, is this: that the fructification (or cone) of *Bennettites Gibsonianus*, Carr., is to be regarded as *more primitive in structure than that of modern Cycads* owing to the fact that its sporophylls possess both a *radial* symmetry of structure and (what is, probably, a necessary condition) bear the ovules in a *terminal* position. This view of the matter will save the incongruity of a plant, like the one under consideration, possessing a vegetative structure (as of the stem) considerably more primitive and a fructification much more advanced than that of modern Cycads. And I would further point out that *complexity* of differentiation in a structure (as in the cone of *Bennettites*) is by no means necessarily synonymous with an *advance* in *evolutionary* development, and this for the reasons above given.

In conclusion, the relationships between the structure of the cone of *Bennettites*, of the cone of modern Cycads, and of *Ginkgo*, may be indicated, on the lines laid down above, in tabular form, thus:—



BENNETTITES.	MODERN CYCADS.	GINKGO.
<i>Female Cone.</i>	<i>Female Cone.</i>	<i>Female Cone.</i>
Axis extremely short and flattened.	Axis much elongated.	Axis much elongated.
Linear-lanceolate bracts permanently ensheathing entire cone.	Linear-lanceolate bracts ensheathing entire cone when young.	
Sporophylls radial in structure.	Sporophylls usually radial or subradial in structure (that of <i>Cycas</i> dorsiventral, but its ovuliferous segments radial).	Sporophylls when (as in abnormalities) developed, radial in structure.
Ovules terminal on sporophyll.	Ovules marginal or subdorsal on sporophyll (those of <i>Cycas</i> terminal to segments of sporophyll).	Ovules terminal on sporophyll.
Sporophylls terminal and sub-terminal to axis of cone.	Sporophylls mostly lateral, upper ones terminal to axis of cone.	Sporophylls both terminal and lateral to axis of cone.
Interstitial organs (sterile sporophylls?) between the fertile sporophylls which overtop and envelop latter.	Interstitial organs absent; protection afforded by swollen terminal portion of each fertile sporophyll.	Ovules exposed, without any protective covering.
Two fleshy, hypogaeal cotyledons; very little endosperm to be seen.	Two fleshy, hypogaeal cotyledons embedded in endosperm of seed.	Two fleshy, hypogaeal cotyledons embedded in endosperm of seed.

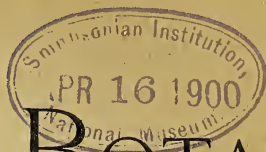
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KEW, June 29, 1900.



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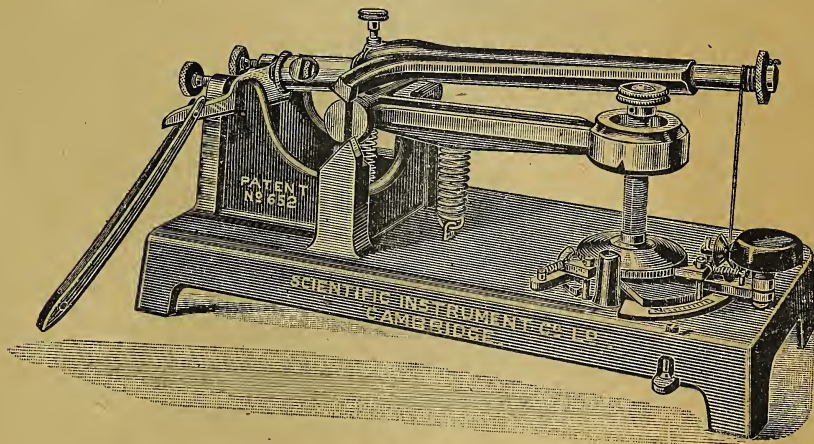
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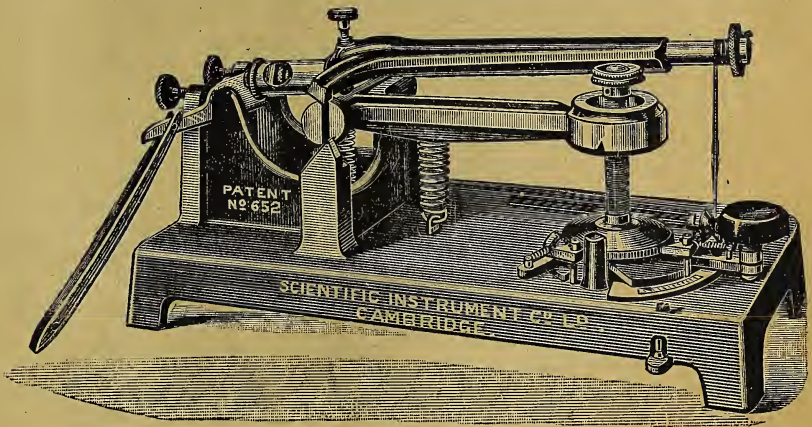


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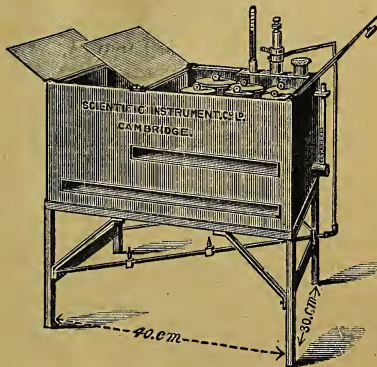


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# ANNALS OF BOTANY

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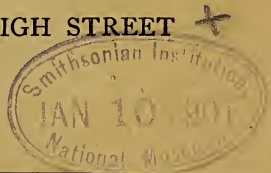
London

HENRY FROWDE, AMEN CORNER, E.C.

Oxford

CLARENDON PRESS DEPOSITORY, 116 HIGH STREET

1900





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